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(54) HI7T213 TRANSGENIC ANIMAL, NEW HI7T213 PROTEIN AND DNA OF THE SAME

(57)Abstract:

PROBLEM TO BE SOLVED: To provide an HI7T213 transgenic non-human mammal, to provide a new HI7T213 protein, to provide a DNA encoding the protein, and to provide uses thereof.

SOLUTION: This transgenic non-human mammal capable of being utilized as a pathological model animal for cataract and other diseases and capable of conducting clarification of pathological mechanisms of the diseases, investigation of a therapeutic method of the diseases, screening of a prophylactic and/or remedy for the diseases, a screening method using the transgenic non-human mammal, the HI7T213 protein, a method for producing the the HI7T213 protein by using a transformant, an antibody of the same, and the DNA encoding the protein are provided, respectively.

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## LEGAL STATUS

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[Date of extinction of right]

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**CLAIMS**

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[Claim(s)]

[Claim 1]

The nonhuman mammal which has DNA incorporating foreignness HI7T213 gene or its mutant allele, or its part.

[Claim 2]

The animal according to claim 1 whose nonhuman mammal is a rat, or its part.

[Claim 3]

foreignness HI7T213 gene -- array number: -- the animal according to claim 1 which is the gene which carries out the code of the HI7T213 which have the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, or its part.

[Claim 4]

foreignness HI7T213 gene -- array number: -- the animal according to claim 1 which is the gene which carries out the code of the Homo sapiens origin HI7T213 which consist of an amino acid sequence expressed with 3, or its part.

[Claim 5]

- (1) The symptoms of a cataract are shown,
- (2) The symptoms of rough hair are shown,
- (3) A transient skin exanthema or desquamation is accepted,
- (4) In an eyeball, fusion/denaturation of the fibrae lentis are accepted,
- (5) The abnormalities of the fibrae lentis are accepted in an eyeball,
- (6) Stratification of the vortex lentis in the stella lentis iridica is seen,
- (7) The basophilia renal tubule is increasing with the kidney,
- (8) The cell damage has occurred with the kidney,
- (9) The growth promotion activity of a tubular cell is high,
- (10) The acanthosis and parakeratosis are accepted in the skin of a juvenile

period,

(11) The PCNA positivity cell which is a growth related antigen is accepted the stratum basale epidermidis and near pore,

(12) The increment in a keratin 6 gene-expression cell or the increment in a PCNA positivity cell is accepted,

(13) Cell proliferation promotion activity is accelerating,

(14) A keratin 14, a keratin 10, or the abnormalities in epidermal differentiation accompanied by manifestation sthenia of RORIKURIN are accepted,

(15) In a keratin 6 positivity part, an epidermis free nerve ending is abundant,

(16) the manifestation of the neurotrophic factor group in the abnormality part in epidermis is reinforcing -- and

(17) The abnormalities in differentiation accompanying growth promotion activity,

since -- an animal given in four from claim 1 which shows at least one phenotype chosen, or its part.

[Claim 6]

The screening approach of HI7T213 agonist characterized by applying an examined substance to an animal given in either of claims 1-4, or its part, and authorizing HI7T 213 agonist activity or HI7T 213 antagonist activity, or HI7T213 antagonist.

[Claim 7]

The screening approach of the matter used for prevention of the cancer and renal dysfunction which apply an examined substance to an animal given in either of claims 1-5, or its part, and are characterized by authorizing the improvement effect of phenotype according to claim 5 or cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, or a hyperalgesia, a cataract, dermatitis, a chronic pain, or a hyperalgesia, and/or a therapy.

[Claim 8]

An animal given in either of claims 1-4 for screening the matter used for prevention and/or the therapy of a wound or injury of spinal cord, or kidney playback, or a part of its use.

[Claim 9]

An animal given in either of claims 1-5 for screening the matter used for prevention of cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, or a hyperalgesia, and/or a therapy, or a part of its use.

[Claim 10]

The fertilized egg which introduced foreignness HI7T213 gene or its mutant allele.

[Claim 11]

The vector which contains foreignness HI7T213 gene or its mutant allele, and may discover this gene in a nonhuman mammal.

[Claim 12]

foreignness HI7T213 gene -- array number: -- the vector according to claim 11 which is the gene which carries out the code of the Homo sapiens origin HI7T213 which consist of an amino acid sequence expressed with 3.

[Claim 13]

The vector according to claim 11 displayed by pCAG 213-1.

[Claim 14]

The transformant by which the transformation was carried out by the vector according to claim 11.

[Claim 15]

The transformant according to claim 14 whose transformant is Escherichia coli JM109/pCAG 213-1 (FERM BP-8207).

[Claim 16]

Array number: The G-protein conjugation mold receptor protein characterized by containing the same amino acid sequence identically to the amino acid sequence expressed with 5, or substantially, its partial peptide, or its salt.

[Claim 17]

Array number: The G-protein conjugation mold receptor protein according to claim 16 which consists of an amino acid sequence expressed with 5, or its salt.

[Claim 18]

The polynucleotide containing the polynucleotide which carries out the code of G-protein conjugation mold receptor protein according to claim 16 or its partial peptide.

[Claim 19]

The polynucleotide according to claim 18 which is DNA.

[Claim 20]

Array number: DNA which consists of a base sequence expressed with 6.

[Claim 21]

The recombination vector containing a polynucleotide according to claim 18.

[Claim 22]

The transformant which carried out the transformation by the recombination vector according to claim 21.

[Claim 23]

The manufacturing method of the G-protein conjugation mold receptor protein according to claim 16 characterized by cultivating a transformant according to claim 22 and making G-protein conjugation mold receptor protein according to claim 16 or its partial peptide generate, its partial peptide, or its salt.

[Claim 24]

Array number: Prevention and/or the therapy agent of the wound or injury of

spinal cord which comes to contain the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, or its salt, or a kidney regenerant.

[Claim 25]

Array number: Prevention and/or the therapy agent of the wound or injury of spinal cord which comes to contain the polynucleotide containing the polynucleotide which carries out the code of the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, or its partial peptide, or a kidney regenerant.

[Claim 26]

Array number: The wound which comes to contain the polynucleotide containing the polynucleotide which carries out the code of the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, or its partial peptide, injury of spinal cord, cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, or the diagnostic agent of a hyperalgesia.

[Claim 27]

The antibody to G-protein conjugation mold receptor protein according to claim 16, its partial peptide, or its salt.

[Claim 28]

The antibody according to claim 27 which is a neutralizing antibody which inactivates signal transfer of G-protein conjugation mold receptor protein according to claim 16.

[Claim 29]

Array number: Prevention and/or the therapy agent of the cancer which comes to contain the antibody to the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, its partial peptide, or its salt, renal dysfunction, a cataract, dermatitis, a chronic pain, or a hyperalgesia.

[Claim 30]

Array number: The wound which comes to contain the antibody to the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, its partial peptide, or its salt, injury of spinal cord, cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, or the diagnostic agent of a hyperalgesia.

[Claim 31]

The polynucleotide which comes to contain a polynucleotide according to claim 18, a complementary base sequence, or its part.

[Claim 32]

Array number: Prevention and/or the therapy agent of the cancer which comes to contain the polynucleotide which comes to contain the polynucleotide containing the polynucleotide which carries out the code of the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, or its partial peptide, a complementary base sequence, or its part, renal dysfunction, a cataract, dermatitis, a chronic pain, or a hyperalgesia.

[Claim 33]

Array number: Prevention and/or the therapy agent of the wound or injury of spinal cord which comes to contain the ligand or agonist to the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, its partial peptide, or its salt, or a kidney regenerant.

[Claim 34]

Array number: Prevention and/or the therapy agent of the cancer which comes to contain the antagonist to the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, its partial peptide, or its salt, renal dysfunction, a cataract, dermatitis, a chronic pain, or a hyperalgesia.

[Claim 35]

Array number: Prevention and/or the therapy agent of the wound or injury of spinal cord which comes to contain the matter to which the amount of manifestations of the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7 or substantially, or its partial peptide is made to increase, or a kidney regenerant.

[Claim 36]

Array number: Prevention and/or the therapy agent of the cancer which comes to contain the matter which decreases the amount of manifestations of the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, or its partial peptide, renal dysfunction, a cataract, dermatitis, a chronic pain, or a hyperalgesia.

[Claim 37]



the G-protein conjugation mold receptor protein which contains the same amino acid sequence to mammalian identically to the amino acid sequence expressed with (i) array number:1, array number:3, array number:5, or array number:7, or substantially — The partial peptide or its salt, (ii) — array number: — the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, or its partial peptide The polynucleotide containing the polynucleotide which carries out a code, array (iii) number:1, array number:3, array number:5, or an array number : The G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 7, or substantially, Ligand or agonist to the partial peptide or its salt, Or (iv) array number : The G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, The prevention and/or the cure for the wound or injury of spinal cord characterized by prescribing for the patient the effective dose of the matter to which the amount of manifestations of the partial peptide or its salt is made to increase, or the kidney playback approach.

[Claim 38]

the G-protein conjugation mold receptor protein which contains the same amino acid sequence to mammalian identically to the amino acid sequence expressed with (i) array number:1, array number:3, array number:5, or array number:7, or substantially — The antibody to the partial peptide or its salt, (ii) — array number: — the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, or its partial peptide The polynucleotide which comes to contain the polynucleotide containing the polynucleotide which carries out a code, a complementary base sequence, or its part, Array number : The G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, (iii) The antagonist to the partial peptide or its salt or (iv) array number:1, array number:3, array number:5, or an array number : The G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 7, or substantially, The prevention and/or the cure for the cancer characterized by prescribing for the patient the effective dose of the matter which decreases the amount of manifestations of the partial peptide or its salt, renal dysfunction, a cataract, dermatitis, a chronic pain, or a hyperalgesia.

**[Claim 39]**

In order to manufacture prevention and/or the therapy agent of a wound or injury of spinal cord, or a kidney regenerant, (i) — array number: — the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially — The partial peptide or its salt, (ii) — array number: — the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, or its partial peptide The polynucleotide containing the polynucleotide which carries out a code, array (iii) number:1, array number:3, array number:5, or an array number : The G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 7, or substantially, Ligand or agonist to the partial peptide or its salt, Or (iv) array number : The G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, Use of the matter to which the amount of manifestations of the partial peptide or its salt is made to increase.

**[Claim 40]**

In order to manufacture prevention and/or the therapy agent of cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, or a hyperalgesia, (i) — array number: — the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially — The antibody to the partial peptide or its salt, (ii) — array number: — the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, or its partial peptide The polynucleotide which comes to contain the polynucleotide containing the polynucleotide which carries out a code, a complementary base sequence, or its part, Array number : The G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, (iii) The antagonist to the partial peptide or its salt or (iv) array number:1, array number:3, array number:5, or an array number : The G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 7, or substantially, Use of the matter which decreases the amount of manifestations of the partial peptide or its salt.

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[Translation done.]

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**DETAILED DESCRIPTION**

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[Detailed Description of the Invention]

[Field of the Invention]

[0001]

This invention relates to those applications etc. at the DNA list which carries out the code of a HI7T 213 transgenics nonhuman mammal and new HI7T213 protein, and this protein.

[Background of the Invention]

[0002]

A cell receives important functional accommodation of accommodation of maintenance of a living body's homeostasis, reproduction, development of an individual, a metabolic turnover, growth, a nervous system, the circulatory system, an immune system, a digestive system, and a metabolic system, feeling acceptance, etc. through the specific receptor which exists in the cell membrane which the living body equips with the feeling stimulus of various hormone, the internality factor or light like neurotransmitter, a smell, etc. to these, and it is performed by carrying out the reaction according to it. It is characterized by for many of receptors of hormone or neurotransmitter which participate in such functional accommodation conjugating with guanine nucleotide-binding protein (it being hereafter called G-protein for short), transmitting a signal to intracellular by activation of this G-protein, and making various functions discover. Moreover, these receptor protein has seven transmembrane domains in common. Such a receptor is named generically a G-protein conjugation mold receptor or a 7 times film penetration mold receptor from these things. Thus, although it turns out that various hormone, neurotransmitter, and the receptor protein to it existed in accommodation of a living body function, it interacted, and the important role is played, there are many still unknown things about whether the receptor to strange active substances (hormone, neurotransmitter, etc.) and strange it exists.

In recent years, the human gene has been solved at an increasing tempo by are recording of the array information by the human genome DNA or random sequencing of cDNA of the various human tissue origins, and rapid advance of a gene-analysis technique. In connection with it, existence of many genes expected to carry out the code of the protein of functional strangeness is clear. Since many consensus sequences exist in the nucleic acid or amino acid, it a G-protein conjugation mold

receptor not only has seven transmembrane domains, but is clearly classifiable as a G-protein conjugation mold receptor out of such protein. on the other hand, the polymerase chain reaction (it is called PCR for short below Polymerase Chain Reaction:) using the similarity of such structure — such a G-protein conjugation mold receptor gene is obtained also by law. Thus, although it may be a subtype with the homology of structure with a known receptor high in the inside of the G-protein conjugation mold receptor obtained until now and it may be possible to predict the ligand easily, the ligand to which the internality ligand cannot be predicted in almost all cases, and these receptors correspond is not found out. These receptors are called the orphan receptor from this. Since ligand was not known, such endogenous ligand that is not identified [ of an orphan receptor ] may be participating in the living thing phenomenon in which sufficient analysis was not made. And when such ligand is connected with an important physiological function and symptoms, it is expected that development of the receptor agonist or an antagonist will be connected with the invention of innovative drugs (Stadel, J.et al., TIPS, 18 volumes, 430 – 437 pages, 1997, Marchese, A).

et al., TIPS, 20 volumes, 370 – 375 pages, 1999, Civelli, O.et al., Brain Res., 848 volumes, 63 – 65 pages, 1999. However, there are not so many examples which actually identified the ligand of an orphan G-protein conjugation mold receptor until now.

Recently, the attempt of ligand retrieval of such an orphan receptor is made by some groups, and isolation and structure determination of the ligand which is new bioactive peptide are reported. Reinsheid et al., Meunier and others introduce independently cDNA which carries out the code of the orphan G-protein conjugation mold receptor LC 132 or ORL1 to an animal cell, and make a receptor discover. The new peptide named orphanin FQ or nociceptin by making the response into an index is isolated from the extract of the Buta brain or a rat brain. The array was determined (Reinsheid, R.K.et al., Science, 270 volumes, 792 – 794 pages, 1995, Meunier, J.-C.et al., Nature, 377 volumes, 532 – 535 pages, 1995). Although it was reported that this peptide is participating in nociception, it was shown clearly further that it was participating in storage by research of the knockout mouse of a receptor (Manabe, T.et al., Nature, 394 volumes, 577 – 581 pages, 1998).

By the approach same after that until now as the above, PrRP (prolactin releasing peptide), new peptides, such as orexin, apelin, ghrelin, and GALP (galanin-like peptide), were isolated as ligand of an orphan G-protein conjugation mold receptor (it Nature(s) Hinuma and S.et al. —) 393 volumes, 272 – 276 pages, 1998, Sakurai, T.et al., Cell, 92 volumes, 573 – 585 pages, 1998, Tatemoto, K.etal., Bichem.Biophys.Res.Comm., 251 volumes, 471 – 476 pages, 1998, Kojima, M.et al., Nature, 402 volumes, 656 – 660 pages, 1999, Ohtaki, T.et al., J.Biol.Chem., 274 volumes, 37041 – 37045 pages, 1999.

The receptor of the bioactive peptide which was not clear on the other hand until now may be solved by the same approach. it was shown clearly that the receptor of motilin which participates in intestinal tract contraction was GPR38 (Feighner and S.D.et al. —) Others [ 1999 / Science, 284 volumes, 2184 – 2188 pages, and ], SLC-1 identifies as a receptor of melanin-concentrating hormone (MCH) — having (Chambers and J.et al. —) Nature, 400 volumes, 261 – 265 pages, 1999, Saito, Y.et

al., Nature, 400 volumes, 265 – 269 pages, 1999, Shimomura, Y.et al., Biochem.Biophys.Res.Comm., 261 volumes, 622 – 626 pages, 1999, Lembo, P.M.C.et al., Nature Cell Biol., one volume, 267 – 271 pages, 1999, Bachner, D.et al., FEBS Lett., 457 volumes, it was reported that 522 – 524 pages, 1999, and GPR14 (SENR) are the receptors of urotensin II (Ames and R.S.et al. —) Nature, 401 volumes, 282 – 286 pages, 1999, Mori, M.et al., Biochem.Biophys.Res.Comm., 265 volumes, 123 – 129 pages, 1999, Nothacker, H.-P.et al., Nature Cell Biol., one volume, 383 – 385 pages, 1999, Liu, Q.et al., Biochem.Biophys.Res.Comm., 266 volumes, 174 – 178 pages, 1999. Although participating in obesity was shown since the knockout mouse showed the phenotype of emaciation (Shimada, M.et al., Nature, 396 volumes, 670 – 674 pages, 1998), the retrieval of the receptor antagonist which has the possibility as antiadipositacs of MCH was attained by having clarified the receptor. Moreover, since cardiac ischemia is caused by administering urotensin II intravenously to an ape, it is also reported that a powerful operation is shown in the cardiac circulatory system (Ames, R.S.et al., Nature, 401 volumes, 282 – 286 pages, 1999).

Thus, an orphan receptor and its ligand participate in a new physiological function in many cases, and it is expected that the elucidation will be connected with new drugs development. However, many difficulties follow in ligand retrieval of an orphan receptor, and while existence of many orphan receptors is clarified until now, it does not pass over the receptor by which the ligand was clarified very in part.

hHI7T213 are known as one of the orphan receptors (1 and an array number: array number : 3; JP,2000-166576,A (patent reference 1)). Moreover, MrgX3 (Homo sapiens mold), MrgA4 (mouse mold), and MrgA6 (mouse mold) are known as an orphan receptor (Nature Neuroscience, 5, 201-209 (nonpatent literature 1 (2002)), Cell, 106, 619-632 (nonpatent literature 2) (2001)), and this MrgX3 has the same amino acid sequence as hHI7T213 (array number: 1). Furthermore, as for hHI7T213, the gun gene mas has about 30% of homology on amino acid level.

[Patent reference 1] JP,2000-166576,A

[Nonpatent literature 1] Nature Neuroscience, 5, 201-209 (2002)

[Nonpatent literature 2] Cell, 106, 619-632 (2001)

[Description of the Invention]

[Problem(s) to be Solved by the Invention]

[0003]

A new transgenics (transgenic) animal enables development of the new drugs which are useful to prevention and the therapy of various diseases.

therefore — the field of this invention — a HI7T 213 transgenics nonhuman animal (a transgenic animal may be called hereafter) — a header, cancer, a cataract, dermatitis, a chronic pain, or nociception — development of the approach of producing which sensitive animal used in disease modeling in large quantities was desired.

[Means for Solving the Problem]

[0004]

In order to solve the above-mentioned technical problem, as a result of repeating research wholeheartedly, when this invention persons produced the new transgenic mouse which made HI7T213 of foreignness discover, they found out that phenotypes,

such as a cataract, rough hair, a skin exanthema, and desquamation, were shown. Furthermore, this invention persons succeeded in carrying out cloning of the new cDAN which carries out the code of the new mouse origin HI7T213 (array number: 5) from which well-known MrgA4 and some amino acid differ, respectively. this invention person came to complete this invention, as a result of basing these knowledge and repeating examination further.

Namely, this invention

[1] The nonhuman mammal which has DNA incorporating foreignness HI7T213 gene or its mutant allele, or its part (living body),

[2] An animal or its part of the above-mentioned [1] publication whose nonhuman mammal is a rat

[3] foreignness -- HI -- seven -- T -- 213 -- a gene -- an array -- a number -- : -- one -- an array -- a number -- : -- three -- an array -- a number -- : -- five -- or -- an array -- a number -- : -- seven -- expressing -- having -- an amino acid sequence -- the same -- or -- substantial -- being the same -- an amino acid sequence -- having -- HI -- seven -- T -- 213 -- a code -- carrying out -- a gene -- it is -- the above -- [ -- one -- ] -- a publication -- an animal -- or -- the -- a part

[4] foreignness HI7T213 gene -- array number: -- the Homo sapiens origin HI7T above-mentioned [1] publication it is unstated from the amino acid sequence expressed with 3 and which is the gene which carries out the code of 213 -- an animal -- or -- the -- a part

The symptoms of [5 (1)] cataracts are shown,

(2) The symptoms of rough hair are shown,

(3) A transient skin exanthema or desquamation is accepted,

(4) In an eyeball, fusion/denaturation of the fibrae lentis are accepted,

(5) The abnormalities of the fibrae lentis are accepted in an eyeball,

(6) Stratification of the vortex lentis in the stella lentis iridica is seen,

(7) The basophilia renal tubule is increasing with the kidney,

(8) The cell damage has occurred with the kidney,

(9) The growth promotion activity of a tubular cell is high,

(10) The acanthosis and parakeratosis are accepted in the skin of a juvenile period,

(11) The PCNA positivity cell which is a growth related antigen is accepted the stratum basale epidermidis and near pore,

(12) The increment in a keratin 6 gene-expression cell or the increment in a PCNA positivity cell is accepted,

(13) Cell proliferation promotion activity is accelerating,

(14) A keratin 14, a keratin 10, or the abnormalities in epidermal differentiation accompanied by manifestation sthenia of RORIKURIN are accepted,

(15) In a keratin 6 positivity part, an epidermis free nerve ending is abundant,

(16) the manifestation of the neurotrophic factor group in the abnormality part in epidermis is reinforcing -- and

(17) The abnormalities in differentiation accompanying growth promotion activity, since -- the animal given [ the above [1] to ] in [4] which shows at least one phenotype chosen, or its part,

[6] The screening approach of HI7T213 agonist characterized by applying an

examined substance to an animal given in either of [4], or its part from the above [1], and authorizing HI7T 213 agonist activity or HI7T 213 antagonist activity, or HI7T213 antagonist,

[7] HI7T213 agonist in which it is obtained by the screening approach of the above-mentioned [6] publication, and deals,

[8] The wound which comes to contain HI7T213 agonist in which it is obtained by the screening approach of the above-mentioned [6] publication, and deals, injury of spinal cord or prevention of the analgesia and/or a therapy agent, or a kidney regenerant,

[9] an animal given in either of the above [1] to [5], or its part -- an examined substance -- applying -- the phenotype of the above-mentioned [5] publication or cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, or nociception -- the cancer and the renal dysfunction which are characterized by to authorize a sensitive improvement effect, a cataract, dermatitis, a chronic pain, or nociception -- the screening approach of the matter used for sensitive prevention and/or a therapy,

[10] Matter used for prevention of the cancer and renal dysfunction which are acquired using the screening approach of the above-mentioned [9] publication, a cataract, dermatitis, a chronic pain, or a hyperalgesia, and/or a therapy,

[11] the cancer which comes to contain the matter obtained using the screening approach of the above-mentioned [9] publication, renal dysfunction, a cataract, dermatitis, a chronic pain, or nociception -- the sensitive physic for prevention and/or a therapy,

[12] The wound characterized by prescribing for the patient the effective dose of HI7T213 agonist obtained using the screening approach of the above-mentioned [6] publication to mammalian, injury of spinal cord or prevention of the analgesia and/or a cure, or the kidney playback approach,

[13] Use of HI7T213 agonist obtained using the screening approach of the above-mentioned [6] publication for manufacturing a wound, injury of spinal cord or prevention of the analgesia and/or a therapy agent, or a kidney regenerant,

[14] An animal given in either of the above [1] for screening the matter used for a wound, injury of spinal cord or prevention of the analgesia and/or a therapy, or kidney playback to [4], or a part of its use,

[15] the cancer characterized by prescribing for the patient the effective dose of the matter obtained using the screening approach of the above-mentioned [9] publication to mammalian, renal dysfunction, a cataract, dermatitis, a chronic pain, or nociception -- sensitive prevention and/or a sensitive cure,

Use of the matter obtained using [16] cancers, renal dysfunction, a cataract, dermatitis, a chronic pain, or the screening approach given in nociception above-mentioned [ for manufacturing sensitive prevention and/or a therapy agent ] [9].

An animal given in either of the above [1] for screening the matter used for prevention of [17] cancers, renal dysfunction, a cataract, dermatitis, a chronic pain, or a hyperalgesia, and/or a therapy to [5], or a part of its use,

[18] The fertilized egg which introduced foreignness HI7T213 gene or its mutant allele,

[19] The vector which contains foreignness HI7T213 gene or its mutant allele, and may discover this gene in a nonhuman mammal,



[20] foreignness HI7T213 gene — array number: — the Homo sapiens origin HI7T above-mentioned [19] publication it is unstated from the amino acid sequence expressed with 3 and which is the gene which carries out the code of 213 — a vector

A vector given in [21] above-mentioned [ containing the CAG promotor who contains a fowl actin promotor further, the field containing a rabbit globin poly A addition signal, an SV40 duplicate initiation field, an ampicillin resistance gene, and a neomycin resistance gene ] [19],

[22] The vector of the above-mentioned [19] publication displayed by pCAG 213-1,

[23] The transformant by which the transformation was carried out by the vector of the above-mentioned [19] publication,

[24] The transformant of the above-mentioned [23] publication whose transformant is Escherichia coli JM109/pCAG 213-1 (FERM BP-8207),

[25] Array number : the G-protein conjugation mold receptor protein characterized by containing the same amino acid sequence identically to the amino acid sequence expressed with 5, or substantially, or its salt,

[26] Array number : the G-protein conjugation mold receptor protein or its salt of the above-mentioned [25] publication it is unstated from the amino acid sequence expressed with 5,

[27] The partial peptide or its salt of the G-protein conjugation mold receptor protein of the above-mentioned [25] publication,

[28] The polynucleotide containing the polynucleotide which carries out the code of the G-protein conjugation mold receptor protein or its partial peptide of the above-mentioned [25] publication,

[29] The polynucleotide of the above-mentioned [28] publication which is DNA,

[30] Array number : DNA which consists of a base sequence expressed with 6,

[31] The recombination vector containing the polynucleotide of the above-mentioned [28] publication,

[32] The transformant which carried out the transformation by the recombination vector of the above-mentioned [31] publication,

[33] The G-protein conjugation mold receptor protein of the above-mentioned [25] publication characterized by cultivating the transformant of the above-mentioned [32] publication and making the G-protein conjugation mold receptor protein or its partial peptide of the above-mentioned [25] publication generate, its partial peptide, or the manufacturing method of the salt,

[34] Physic which comes to contain the G-protein conjugation mold receptor protein of the above-mentioned [25] publication, its partial peptide, or its salt,

[35] Physic which comes to contain the polynucleotide of the above-mentioned [28] publication,

[36] Array number : the wound which comes to contain the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, or its salt, injury of spinal cord or prevention of the analgesia and/or a therapy agent, or a kidney regenerant,

[37] Array number : the wound which comes to contain the polynucleotide containing the polynucleotide which carries out the code of the G-protein conjugation mold

receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, or its partial peptide, injury of spinal cord or prevention of the analgesia and/or a therapy agent, or a kidney regenerant,

[38] array number: -- the wound which comes to contain the polynucleotide containing the polynucleotide which carries out the code of the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, or its partial peptide, injury of spinal cord, the analgesia, cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, or nociception -- a sensitive diagnostic agent,

[39] The G-protein conjugation mold receptor protein of the above-mentioned [25] publication, its partial peptide, or the antibody to the salt,

[40] The antibody of the above-mentioned [39] publication which is the neutralizing antibody which inactivates signal transfer of the G-protein conjugation mold receptor protein of the above-mentioned [25] publication,

[41] Physic which comes to contain the antibody of the above-mentioned [39] publication,

[42] array number: -- the cancer which comes to contain the antibody to the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, its partial peptide, or its salt, renal dysfunction, a cataract, dermatitis, a chronic pain, or nociception -- sensitive prevention and/or a sensitive therapy agent,

[43] array number: -- the wound which comes to contain the antibody to the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, its partial peptide, or its salt, injury of spinal cord, the analgesia, cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, or nociception -- a sensitive diagnostic agent,

[44] The polynucleotide which comes to contain the polynucleotide of the above-mentioned [28] publication, a complementary base sequence, or its part,

[45] Physic which comes to contain the polynucleotide of the above-mentioned [44] publication,

[46] array number: -- the cancer which comes to contain the polynucleotide which comes to contain the polynucleotide containing the polynucleotide which carries out the code of the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, or its partial peptide, a complementary base sequence, or its part, renal dysfunction, a cataract, dermatitis, a chronic pain, or nociception -- sensitive prevention and/or a sensitive therapy agent,

[47] The compound to which affinity with the ligand characterized by using the G-protein conjugation mold receptor protein of the above-mentioned [25] publication, its partial peptide, or its salt, this G-protein conjugation mold receptor protein, its partial peptide, or its salt is changed, or the screening approach of the

salt,

[48] The compound to which affinity with the ligand characterized by containing the G-protein conjugation mold receptor protein of the above-mentioned [25]

publication, its partial peptide, or its salt, this G-protein conjugation mold receptor protein, its partial peptide, or its salt is changed, or the kit for screening of the salt,

[49] The compound to which affinity with the ligand and this G-protein conjugation mold receptor protein in which it obtains and deals using the screening approach of the above-mentioned [47] publication or the kit for screening of the

above-mentioned [48] publication, its partial peptide, or its salt is changed, or its salt,

[50] Physic which comes to contain the compound of the above-mentioned [49] publication, or its salt,

[51] Array number : the wound which comes to contain the ligand or agonist to the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, its partial peptide, or its salt, injury of spinal cord or prevention of the analgesia and/or a therapy agent, or a kidney regenerant,

[52] array number: — the cancer which comes to contain the antagonist to the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, its partial peptide, or its salt, renal dysfunction, a cataract, dermatitis, a chronic pain, or nociception — sensitive prevention and/or a sensitive therapy agent,

[53] The screening approach of the matter to which the amount of manifestations of the G-protein conjugation mold receptor protein of the above-mentioned [25] publication characterized by using DNA of the above-mentioned [29] publication or its partial peptide is changed,

[54] The kit for screening of the matter to which the amount of manifestations of the G-protein conjugation mold receptor protein of the above-mentioned [25] publication characterized by containing DNA of the above-mentioned [29] publication or its partial peptide is changed,

[55] Matter to which the amount of manifestations of the G-protein conjugation mold receptor protein of the above-mentioned [25] publication in which it obtains and deals using the screening approach of the above-mentioned [53] publication or the kit for screening of the above-mentioned [54] publication, or its partial peptide is changed,

[56] Array number : the wound which comes to contain the matter to which the amount of manifestations of the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7 or substantially, or its partial peptide is made to increase, injury of spinal cord or prevention of the analgesia and/or a therapy agent, or a kidney regenerant,

[57] array number: — the cancer which comes to contain the matter which decreases the amount of manifestations of the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or

substantially, or its partial peptide, renal dysfunction, a cataract, dermatitis, a chronic pain, or nociception — sensitive prevention and/or a sensitive therapy agent,

[58] the G-protein conjugation mold receptor protein which contains the same amino acid sequence to mammalian identically to the amino acid sequence expressed with (i) array number:1, array number:3, array number:5, or array number:7, or substantially, its partial peptide, or its salt — (ii) — array number: — the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, or its partial peptide The polynucleotide containing the polynucleotide which carries out a code, array (iii) number:1, array number:3, array number:5, or an array number : The G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 7, or substantially, Ligand or agonist to the partial peptide or its salt, Or (iv) array number : The G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, The wound characterized by prescribing for the patient the effective dose of the matter to which the amount of manifestations of the partial peptide or its salt is made to increase, injury of spinal cord or prevention of the analgesia and/or a cure, or the kidney playback approach,

[59] the G-protein conjugation mold receptor protein which contains the same amino acid sequence to mammalian identically to the amino acid sequence expressed with (i) array number:1, array number:3, array number:5, or array number:7, or substantially — The antibody to the partial peptide or its salt, (ii) — array number: — the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, or its partial peptide The polynucleotide which comes to contain the polynucleotide containing the polynucleotide which carries out a code, a complementary base sequence, or its part, Array number : The G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or substantially, (iii) The antagonist to the partial peptide or its salt or (iv) array number:1, array number:3, array number:5, or an array number : The G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 7, or substantially, The prevention and/or the cure for the cancer characterized by prescribing for the patient the effective dose of the matter which decreases the amount of manifestations of the partial peptide or its salt, renal dysfunction, a cataract, dermatitis, a chronic pain, or a hyperalgesia,

[60] Prevention and/or the therapy agent of a wound, injury of spinal cord, or the analgesia, Or (i) array number:1, array number:3, array number:5, or the array number for manufacturing a kidney regenerant : The G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 7, or substantially, its partial peptide, or its salt, (ii) — array number: — the G-protein conjugation mold receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with 1, array

number:3, array number:5, or array number:7, or substantially, or its partial peptide  
 The polynucleotide containing the polynucleotide which carries out a code, array (iii)  
 number:1, array number:3, array number:5, or an array number : The G-protein  
 conjugation mold receptor protein which contains the same amino acid sequence  
 identically to the amino acid sequence expressed with 7, or substantially, Ligand or  
 agonist to the partial peptide or its salt, Or (iv) array number : The G-protein  
 conjugation mold receptor protein which contains the same amino acid sequence  
 identically to the amino acid sequence expressed with 1, array number:3, array  
 number:5, or array number:7, or substantially, Use of the matter to which the amount  
 of manifestations of the partial peptide or its salt is made to increase,

[61] In order to manufacture prevention and/or the therapy agent of cancer, renal  
 dysfunction, a cataract, dermatitis, a chronic pain, or a hyperalgesia, (i) — array  
 number: — the G-protein conjugation mold receptor protein which contains the same  
 amino acid sequence identically to the amino acid sequence expressed with 1, array  
 number:3, array number:5, or array number:7, or substantially — The antibody to the  
 partial peptide or its salt, (ii) — array number: — the G-protein conjugation mold  
 receptor protein which contains the same amino acid sequence identically to the  
 amino acid sequence expressed with 1, array number:3, array number:5, or array  
 number:7, or substantially, or its partial peptide The polynucleotide which comes to  
 contain the polynucleotide containing the polynucleotide which carries out a code, a  
 complementary base sequence, or its part, Array number : The G-protein conjugation  
 mold receptor protein which contains the same amino acid sequence identically to  
 the amino acid sequence expressed with 1, array number:3, array number:5, or array  
 number:7, or substantially, (iii) The antagonist to the partial peptide or its salt or (iv)  
 array number:1, array number:3, array number:5, or an array number : The G-protein  
 conjugation mold receptor protein which contains the same amino acid sequence  
 identically to the amino acid sequence expressed with 7, or substantially, Use of the  
 matter which decreases the amount of manifestations of the partial peptide or its  
 salt,

\*\*\*\* is offered.

[Effect of the Invention]

[0005]

The transgenics nonhuman mammal of this invention can be used for evaluation of  
 the prophylactic of diseases, such as a cataract, or a remedy, the experiment for  
 gene therapies of a HI7T 213 abnormality-of-the-genes patient, etc., can cultivate  
 the cell extracted from the transgenics nonhuman mammal of this invention, and can  
 use it for evaluation of HI7T213 inhibitor.

[Best Mode of Carrying Out the Invention]

[0006]

The transgenic animal of this invention For example, the fertilized egg of a nonhuman  
 mammal and an unfertilized egg, To a sperm, its precursor cells (a primordial germ  
 cell, oogonium, oocyte, an ootid, spermatogonium, a spermatocyte, spermatid, etc.),  
 etc., it sets preferably at the initial stage (still more preferably 8 cell terms or before)  
 of the embryogenesis of a fertilized egg. a calcium phosphate coprecipitation Mr.  
 method and electric punching (electroporation) — law — the RIPOFE cushion  
 method, a condensation method, and micro impregnation (microinjection) — law and

a gene gun (party Kurgan) — by transgenics methods, such as law and the DEAE-dextran method It is created by introducing into the cell aiming at foreignness HI7T213 target gene or its mutant allele etc. Moreover, by this transgenics method, the target DNA can be introduced into the somatic cell of a nonhuman mammal, an organization, an organ, etc., it can also use for a cell culture, tissue culture, etc., and a transgenic animal can also be further created by uniting this cell with an above-mentioned germ (or reproduction) cell using a well-known cell fusion method. Or the target DNA is introduced into the embryonic stem cell (embryonic stem cell) of a nonhuman mammal like the case where a knock out animal is produced, using the above-mentioned transgenics method. After this DNA chooses beforehand the clone included in stability, inject this embryonic stem cell into a blastocyst, or make an embryonic stem cell lump and 8 cell term germ condense, and a chimeric mouse is produced. It is possible to obtain a transgenic animal also by choosing what the introductory gene was delivered to the germ cell line.

Moreover, some transgenic animals produced by doing in this way (living body) for example, the cell which has DNA incorporating (i) foreignness HI7T213 gene or its mutant allele — The cell or organization originating in these is cultivated. (ii(s)), such as an organization and an organ, — The various protein which can be isolated from this (iii) transgenic animal, such as what carried out the passage, or DNA if needed As "some nonhuman mammals which have DNA incorporating foreignness HI7T213 gene or its mutant allele" of this invention It can use for the same purpose as "the nonhuman mammal which has DNA incorporating foreignness HI7T213 gene or its mutant allele" of this invention.

Especially as an organization which are some transgenic animals, although not limited, the skin, liver, the kidney, the heart, a spleen, lungs, a suprarenal gland, a testis, the ovary, an eyeball, etc. are desirable.

Especially as a cell which are some transgenic animals, although not limited, cells, such as the skin, liver, the kidney, the heart, a spleen, lungs, a suprarenal gland, a testis, the ovary, and an eyeball, are desirable.

[0007]

As target "nonhuman mammal", a cow, Buta, a sheep, a goat, a rabbit, a dog, a cat, a guinea pig, a hamster, a rat, a mouse, etc. are mentioned by this invention.

Preferably, it is a rabbit, a dog, a cat, a guinea pig, a hamster, a mouse, or a rat, and Rodentia (Rodentia) are desirable and they are especially rats (Wistar, SD, etc.) and an object animal as an animal used in disease modeling especially with the most desirable rat of a Wistar network. It can use for the purpose as the "nonhuman mammal" which carries out the object of the fowl etc. by this invention that otherwise it is the same as a birds animal.

As foreignness HI7T213 gene introduced into the target nonhuman mammal, HI7T213 gene of the mammalian origins, such as Homo sapiens, Buta, a sheep, a goat, a rabbit, a dog, a cat, a guinea pig, a hamster, a rat, and a mouse, can be used, for example.

The gene of internality with which the animal for transgenics has foreignness HI7T213 gene is a different gene, and HI7T213 gene or HI7T213 compound gene specifically isolated and refined from the aforementioned mammalian is used.

Specifically as a mutant allele of foreignness HI7T213 gene of this invention, what variation (for example, mutation, site specific mutation, etc.) produced, and the gene

which addition of a base, the deficit, the permutation to other bases, etc. produced are mentioned to DNA of this invention. In the amino acid sequence which more specifically constitutes HI7T213 as a result of addition of this base, a deficit, and the permutation to other bases There may not be 1, and 30 pieces may be which variation as long as it is 1 thru/or the variation which it is still more preferably desirable 1 thru/or to mutate five pieces so that a permutation, addition, or a deficit may arise to 1 or two amino acid more preferably ten pieces, and does not lose the function of HI7T213 preferably.

Although the gene which specifically carries out the code of the HI7T213 mentioned later as foreignness HI7T213 gene, for example is used DNA which carries out the code of the Homo sapiens HI7T213 which more specifically contain the amino acid sequence expressed with array number:1 (array number: 2), Array number : DNA which carries out the code of Homo sapiens HI7T213 containing the amino acid sequence expressed with 3 (array number: 4), Array number: DNA (array number: 6) which carries out the code of mouse HI7T213 containing the amino acid sequence expressed with 5, DNA (array number: 8) which carries out the code of mouse HI7T213 containing the amino acid sequence expressed with array number:7 are used.

[0008]

Although foreignness HI7T213 gene in this invention or its mutant alle (HI7T213 gene may only be called hereafter) may be the thing of the nonhuman mammal made into the object of installation or a manifestation, congener or different species, and which mammalian origin, it is desirable that it is the thing of the mammalian origin of a different kind. If in charge of introducing this gene into an object animal, generally it is advantageous to use as gene constructs (an example, vector, etc.) connected with the lower stream of a river of the promotor who is made discovered in the cell of the target animal and deals in the gene concerned. the various mammals (a rabbit --) which specifically have Homo sapiens HI7T213 gene and HI7T213 gene with high homology when making HI7T213 human gene introduce It originates in a dog, a cat, a guinea pig, a hamster, a rat, a mouse, etc. (preferably rat etc.). By carrying out the microinjection of the vector which connected this gene with the lower stream of a river of the various promoters who are made discovered and deal in HI7T213 human gene to the fertilized egg (for example, rat fertilized egg) of the target nonhuman mammal The transgenics nonhuman mammal which high-discovers Homo sapiens HI7T213 target gene can be created.

As a HI7T 213 gene-expression vector, animal viruses, such as retroviruses, such as bacteriophages, such as a plasmid of the Escherichia coli origin, a plasmid of the Bacillus-subtilis origin, a plasmid of the yeast origin, and lambda phage, and a Moloney leukemia virus, a vaccinia virus, or a baculovirus, etc. are used. Especially, the plasmid of the Escherichia coli origin, the plasmid of the Bacillus-subtilis origin, or the plasmid of the yeast origin is used preferably, and especially the plasmid of the Escherichia coli origin is desirable.

[0009]

As a promotor who performs gene expression accommodation of foreignness HI7T213 gene for example, a virus (a cytomegalovirus and a Moloney leukemia virus --) The promotor of the gene originating in a JC virus, a mammary tumor virus, etc.,

various mammals (Homo sapiens, a rabbit, a dog, a cat, a guinea pig, and a hamster —) The gene originating in birds (fowl etc.), such as a rat and a mouse for example, albumin, endothelin, osteocalcin, and muscle creatine kinase — An I-beam and II mold collagen, a cyclic AMP dependence protein kinase beta subunit, An atrium natriuresis sex factor, a dopamine-beta-hydroxylase, a neurofilament light chain, Metallothioneins I and IIA, METARO proteinase 1 organization inhibitor, Smooth muscle alpha actin and polypeptide chain elongation factor 1alpha (EF1-alpha), beta actin, Promoters, such as alpha and beta myosin heavy chain, myosin light chains 1 and 2, myelin basic protein, a blood serum amyloid P component, and renin, etc. are mentioned, and the CAG promoter who contains a fowl actin promoter especially can be used.

the disease model made into the purpose still more preferably — responding — a target tissue — foreignness HI7T213 gene — specific or the promoter (example: — the blood serum amyloid P component (SAP) in which the high manifestation by liver is possible —) who may make it high-discovered Gene promoters, such as albumin, transferrin, Antithrombin III, and alpha 1-antitrypsin; alpha and beta myosin heavy chain in which a high manifestation with the heart is possible, A myosin light chain 1 And gene promoter [, such as 2, ]; It is a suprarenal gland. Gene promoters, such as a PTH/PTHrP receptor in which a high manifestation with the kidney is possible; The myelin basic protein in which a high manifestation in gene promoter; brains, such as fatty-acid binding protein in which the high manifestation by gene promoter; alimentary canals, such as an ACTH receptor in which a high manifestation is possible, is possible, is possible, Gene promoters, such as glial fiber nature acidic protein, etc. can be chosen suitably. For example, when the transgenic animal of this invention is a kidney disease model, it is desirable to use with the kidney the promoter in whom a high manifestation is possible.

[0010]

as for the above-mentioned vector, it is desirable to have the array (poly A — generally called a terminator) which ends the imprint of mRNA made into the purpose in transgenics mammalian, for example, gene expression can be operated using the array of each gene of the virus origin, various mammals, and the birds origin. Preferably, the SV40 terminator of the simian virus etc. is used. In addition, it is the purpose which makes the target gene high-discover further, and it is also possible by the purpose to connect a part of intron of the splicing signal of each gene, an enhancer field, and an eukaryon gene with 5'3' of between [ the upstream promoterregion, and a translation field ] or translation field' lower stream of a river of promoterregion.

Moreover, as for the above-mentioned vector, it is desirable that a selective marker gene (example: drug resistance genes, such as a neomycin resistance gene, a hygromycin tolerance gene, and ampicillin resistance) for an introductory gene to choose the clone included in stability is included further. Furthermore, when meaning including an introductory gene in the specific part of a host chromosome by homologous recombination (namely, production of a knock in animal), in order to eliminate random insertion, it is desirable [ the above-mentioned vector ] that a herpes simplex virus origin thymidine kinase gene and a diphtheria toxin gene are further included as a negative selective marker gene on a target site and the outside



of a homologous DNA array. These embodiments are explained in full detail later.

[0011]

the translation field of HI7T213 -- Homo sapiens and various nonhuman mammals (a rabbit --) Liver, such as a dog, a cat, a guinea pig, a hamster, a rat, and a mouse, All or a part of genomic DNA originating in DNA originating in the kidney, fibrocyte, etc. and various commercial genomic DNA libraries is used as a raw material. Or it is acquirable from RNA originating in the liver of Homo sapiens or various nonhuman mammals, the kidney, and fibrocyte, using the complementary DNA prepared by the well-known approach as a raw material. Moreover, the translation field which varied by the point mutation inducing method etc. is also producible using the translation field of HI7T213 obtained from an above-mentioned cell or an above-mentioned organization etc. Each of these is ingredients available to a transgenic animal. The above translation field can produce DNA incorporating HI7T213 gene by the usual gene engineering-technique made to connect with the aforementioned promotor's lower stream of a river (preferably upstream like the conclusion section of an imprint) as gene constructs (an example, vector, etc.) which may be discovered in an introductory animal.

pCAG 213-1 (example 1 mentioned later) which inserted HI7T213 gene in the plasmid pCXN2 which specifically has a CAG promotor including a fowl actin promotor, the field containing a rabbit globin poly A addition signal, an SV40 duplicate initiation field, an ampicillin resistance gene, and a neomycin resistance gene is used.

[0012]

The expression vector containing DNA which carries out the code of the desirable foreignness HI7T213 which set like 1 operative condition and are obtained as mentioned above is introduced into the early embryo of the target nonhuman mammal by the microinjection method.

The early embryo of an object nonhuman mammal can be obtained by carrying out the in vitro fertilization of the egg and sperm which extracted the internal fertilization egg which is made to cross the sex of a nonhuman mammal of the same kind, and is obtained, or were extracted from the sex of a nonhuman mammal of the same kind, respectively.

the case where mice (F1 of inbred mice, such as C57BL/6J(B6), desirable B6, and other desirable inbred strains etc.) are used, for example although age, breeding conditions, etc. of a nonhuman mammal of using change with animal species, respectively -- a female -- about 4- that about 6 weeks old and whose male are about 2 - about 8 age-of-the-moon extent is desirable, and what was bred for about one week on about 12-hour \*\* term conditions (for example, 7:00 - 19:00) is desirable.

The approach of internal fertilization of making it cross also by natural mating with the male nonhuman mammal although it is good, after medicating a female nonhuman mammal with a gonadotropic hormone for the purpose of obtaining many early embryos from accommodation of sexual cycle and one individual and carrying out induction of the superovulation is desirable. as an induction-of-ovulation method of a female nonhuman mammal, although introduction follicle-stimulating hormone (pregnant mare serum gonadotropin -- it generally abbreviates to PMSG) and the method of prescribing luteinizing hormone (Homo sapiens chorionic gonadotropin --

it abbreviating to hCG generally) for the patient by intraperitoneal injection etc. are subsequently desirable, the dose of desirable hormone and administration spacing change with classes of nonhuman mammal, respectively, for example. A nonhuman mammal for example, in the case of mice (F1 of inbred mice, such as C57BL/6J(B6), desirable B6, and other desirable inbred strains etc.) Usually, luteinizing hormone is prescribed for the patient follicle-stimulating hormone administration and about 48 hours after. the approach of obtaining a fertilized egg by making it crossing with a male mouse immediately -- desirable -- the dose of follicle-stimulating hormone -- about 20 -- about 50 IU / individual -- desirable -- the dose of about 30 IU / individual, and luteinizing hormone -- about 0 -- about 10 IU / individual -- they are about 5 IU / individual preferably.

The abdominal cavity of the female nonhuman mammal which checked mating by inspection of a vaginal plug etc. is opened after fixed time amount progress, a fertilized egg is taken out from an oviduct, and it washes in the culture media for embryo cultures (example: M16 culture medium, a correction Whitten culture medium, a BWW culture medium, M2 culture medium, a WM-HEPES culture medium, BWW-HEPES culture medium, etc.), and cultivates to DNA micro impregnation by minute drop cultivation etc. except for a cumulus cell under 5% carbon dioxide gas / 95% atmospheric air. When not performing micro impregnation immediately, it is also possible a slow method or overly to carry out cryopreservation by a rapid method etc. in the extracted fertilized egg.

[0013]

On the other hand, in the case of in vitro fertilization, after medicating the female nonhuman mammal for egg gathering (the same thing as the case of internal fertilization is used preferably) with follicle-stimulating hormone and luteinizing hormone like the above and making ovulation induce, an ovum is extracted, and it is a culture medium for fertilization (example: cultivate by minute drop cultivation etc. in a TYH culture medium under 5% carbon dioxide gas / 95% atmospheric air till in vitro fertilization.). On the other hand, the tail-of-epididymidis section is taken out from a male nonhuman mammal (the same thing as the case of internal fertilization is used preferably) of the same kind, a sperm lump is extracted, and preculture is carried out in the culture medium for fertilization. After adding the sperm after preculture termination to the culture medium for fertilization containing an ovum and cultivating by minute drop cultivation etc. under 5% carbon dioxide gas / 95% atmospheric air, the fertilized egg which has two pronuclei is selected under a microscope. When not performing micro impregnation of DNA immediately, it is also possible a slow method or overly to carry out cryopreservation by a rapid method etc. in the obtained fertilized egg.

[0014]

Micro impregnation of DNA to a fertilized egg can be carried out according to a conventional method using well-known equipments, such as a micromanipulator. the fertilized egg put in into the minute drop of the culture medium for embryo cultures when saying briefly -- a holding pipet -- drawing in -- fixing -- an injection pipet -- using -- a DNA solution -- maleness or female pronucleus -- it pours in directly into male pronucleus preferably. As for an introductory gene, it is desirable to use what was refined to altitude by CsCl density gradient ultracentrifuge etc. Moreover, as for

an introductory gene, it is desirable to cut a vector part using a restriction enzyme and to make it the shape of a straight chain.

[0015]

After cultivating the fertilized egg after DNA installation from 1 cell term by minute drop cultivation etc. in the culture medium for embryo cultures at a blastocyst term under 5% carbon dioxide gas / 95% atmospheric air, it is transplanted in the oviduct of the female nonhuman mammal for \*\*\*\* which carried out pseudopregnancy, or a uterus. When the female nonhuman mammal for \*\*\*\* transplants a mouse early embryo with the animal in which the early embryo transplanted originates that what is necessary is just of the same kind, the female mouse (preferably about 8- about 10 weeks old) of an ICR system etc. is used preferably. For example, it is made to cross with a vasectomy (ligation) male nonhuman mammal (in for example, the case of a mouse male mouse of an ICR system (preferably about 2 or more age of the moon)) of the same kind as an approach of changing the female nonhuman mammal for \*\*\*\* into a pseudopregnancy condition, and the approach of choosing that by which existence of a vaginal plug was checked is learned.

\*\*\*\*\* may use the thing of spontaneous ovulation, or in advance of mating with a vasectomy (ligation) male, may prescribe luteinizing hormone releasing hormone (generally it abbreviates to LHRH), or its analog for the patient, and may use that to which induction of the fertility was carried out. As a LHRH analog, [3 and 5-Dil-Tyr5]-LH-RH, [Gln8]-LH-RH, [D-Ala6]-LH-RH, [des-Gly10]-LH-RH, [D-His(Bzl)6]-LH-RH, those Ethylamide(s), etc. are mentioned, for example. A male nonhuman mammal and the stage made to cross change with classes of nonhuman mammal after LHRH or the dose of the analog, and its administration, respectively. for example, the thing which a nonhuman mammal makes cross with a male mouse on about the 4th after usually prescribing LHRH or its analog for the patient in the case of mice (preferably mouse of an ICR system etc.) -- desirable -- the dose of LHRH or its analog -- usually -- about 10-60microg / individual -- they are about 40microg / individual preferably.

[0016]

Usually, when the early embryo transplanted is after a morula term, if it is a front [ it ] (from 1 cell term to for example, 8 cell term germ), an embryo transfer will be carried out to the uterus of \*\*\*\*\* at an oviduct. As for \*\*\*\*\*, that in which a certain days have passed since pseudopregnancy according to the developmental stage of a transplantation germ is used suitably. For example, for the female mouse for about 0.5 days transplanting a blastocyst term germ, the female mouse for about 2.5 days is desirable to transplanting 2 cell term germ in the case of a mouse after pseudopregnancy after pseudopregnancy. After anesthetizing \*\*\*\*\* (Avertin etc. is used preferably), it is cut open, the ovary is pulled out, and the early embryo (about 5- about ten pieces) suspended in the culture medium for embryo cultures is poured in abdominal ostium of uterine tube or near the oviduct joint of a uterine horn using the pipet for embryo transfers.

If a transplantation germ is implanted with the sufficient result and \*\*\*\*\* becomes pregnant, \*\*\*\*\* will be obtained by the natural birth or the cesarean section. When it gives birth by the cesarean section, offspring can be made to suckle the female for breast feeding (for example, female mice to which it crossed and gave

birth usual in the case of the mouse (preferably female mouse of an ICR system etc.)) prepared separately that what is necessary is just to make \*\*\*\*\* which carried out the natural birth continue breast feeding as it is.

[0017]

Installation of the gene which carries out the code of foreignness HI7T213 in an amphicytula phase is secured so that an introductory gene may exist in all the germ cell line cells of an object nonhuman mammal, and somatic cells. It can be authorized by screening the chromosome DNA which carried out the separation extract from the tail of offspring by Southern hybridization or the PCR method whether the introductory gene is included in Chromosome DNA. That the gene which carries out the code of foreignness HI7T213 in the germ cell line cell of \*\*\*\*\* (F0) obtained as mentioned above exists means that the gene to which all the animals of after that cost (F1) carry out the code of foreignness HI7T213 to all the germ cell line cell and somatic cells exists.

Usually, F0 animal is obtained as heterozygote which has an introductory gene only in one side of homologue. Moreover, each F0 individual is inserted at random on a chromosome which is different unless it is based on homologous recombination. What is necessary is to cross F0 animal and a non-transgenic animal, to create F1 animal and just to cross the brothers and sisters of heterozygote which have an introductory gene only in one side of homologue, in order to obtain the homozygote which has the gene which carries out the code of foreignness HI7T213 to both homologues. If the introductory gene is included only in one locus, one fourth of F2 animals obtained will become a homozygote.

[0018]

The expression vector containing another desirable gene which sets like 1 operative condition and carries out the code of foreignness HI7T213 is introduced into the embryonic stem cell (embryonic stem cell) of the target nonhuman mammal by the transgenics method the electroporation method etc. is well-known.

An embryonic stem cell originates in the inner cell mass (ICM) of the fertilized egg of a blastocyst term, and says the cell which can carry out culture maintenance, maintaining an undifferentiated condition by in vitro one. The cell of ICM is a cell which forms a germ body, and will be a stem cell which becomes the radical of all the organizations containing a reproductive cell in the future. What could use the already established cell strain thing as an embryonic stem cell, and was newly established according to the approach (Nature, the 292nd volume, 154 pages, 1981) of Evans and Kaufman may be used. For example, in the case of the mouse embryonic stem cell, the embryonic stem cell of the 129 system mouse origin is used for current and a general target, but For the purpose of a genetic background acquiring a clear embryonic stem cell immunologically by the pure line strain replaced with this, since the immunological background has not clarified for example, the little of C57BL/6 mouse or the number of egg gathering of C57BL/6 — the intersection of DBA/2 — the embryonic stem cell established from BDF1 mouse (F1 of C57BL/6, and DBA/2) improved more coarsely can be used good. the advantage that BDF1 mouse has many egg gathering, and its egg is strong — in addition, since it has against the background of C57BL/6 mouse, the embryonic stem cell of this the origin can be advantageously used at the point which can replace the genetic background with

C57BL/6 mouse by carrying out a back cross to C57BL/6 mouse, when a disease model mouse is created.

[0019]

Preparation of an embryonic stem cell can be performed by [ as being the following ]. When using female nonhuman mammal [ after mating ] [(F1 of inbred mice, such as C57BL/6J(B6), desirable B6, and other desirable inbred strains etc.), for example, mice A blastocyst term germ is extracted from the uterus of] for which the male mouse of about 2 or more age of the moon and the female mouse (about 3.5 days of pregnancy) of about 8 made to cross – about 10 weeks old of abbreviation are used preferably (or after extracting the early embryo before a morula term from an oviduct). you may cultivate like the above in the culture medium for embryo cultures at a blastocyst term, if it cultivates on suitable feeder cell layers (for example, the first fibrocyte, a well-known STO fibrocyte stock, etc. which are prepared from a mouse fetus in the case of a mouse) ICM which some cells of a blastocyst gather and will specialize in a germ in the future is formed. An embryonic stem cell is obtained by repeating dissociation and a passage, carrying out trypsinization of this inner cell mass, making a single cell dissociate, maintaining suitable cell density, and performing culture-medium exchange.

[0020]

an embryonic stem cell — a sex — although any may be used, it is convenient for the direction of a male embryonic stem cell usually creating a germ cell line chimera. Moreover, also in order to reduce the time and effort of complicated culture, it is desirable to distinguish a sex as early as possible. As the judgment approach of the sex of an embryonic stem cell, the approach of amplifying the gene of the sex determination field on a Y chromosome, and detecting by the PCR method, can raise as one of them, for example. If this approach is used, since it will end with the number of embryonic stem cells of 1 colony extent (about 50 pieces) to having taken the number of cells of about 10<sup>6</sup> pieces to carry out karyotype analysis conventionally, it is possible to perform the first selection of the embryonic stem cell in the early stages of culture by distinction of a sex, and the time and effort in early stages of culture can be sharply reduced by having enabled selection of a male cell at an early stage.

Moreover, the check of the chromosome number for example, by the G-banding method etc. can perform as the second selection. Although 100% of the normal number of the chromosome number of the embryonic stem cell obtained is desirable, when a relation top, such as physical actuation in the case of cell strain establishment, is difficult, it is desirable after the transgenics to an embryonic stem cell to carry out cloning to a normal cell (for example, cell whose chromosome number is 2n=40 with a mouse) again.

[0021]

Thus, the embryonic stem cell stock obtained needs to carry out subculture carefully, in order to maintain the property of an undifferentiated stem cell. For example, it is the inside of a carbon-dioxide-gas incubator (preferably) under the LIF (1–10,000U/ml) existence known as differentiation repressor on a suitable feeder cell like STO fibrocyte. It cultivates by the approach of cultivating at about 37 degrees C with 5% carbon dioxide gas / 95% air, or 5% oxygen / 5% carbon dioxide gas / 90% air.

At the time of a passage For example, it single-cell-izes by the trypsin / EDTA solution (usually 0.001 – 0.5% trypsin / 0.1 – 5mM EDTA, preferably about 0.1% trypsin / 1mM EDTA) processing, and the approach of carrying out seeding on the newly prepared feeder cell etc. is taken. Although such a passage is usually performed day by day [ 1 – 3 ], when a cell is observed on this occasion and a cell unusual in gestalt is able to see, to abandon that cultured cell is desired.

By carrying out suspended cell culture until it carries out monolayer culture of it according to suitable conditions until an embryonic stem cell results in high density, or it forms a cell cluster It is possible to make it specialize in the cell of various types, such as a top-of-the-head muscle, a visceral muscle, and a myocardium. [M.J.Evans and M.H.Kaufman, Nature, The 292nd volume, 154 pages, 1981;G.R.Martin, Proc.Natl.Acad.Sci.U.S.A., the 78th volume, 7634 pages, and 1981;T.C.Doetschman \*\* — Journal of embryology and experimental morphology, The foreignness HI7T 213 manifestation nonhuman mammal cell which the embryonic stem cell introduced in the 87th volume, 27 pages, 1985], and the gene that carries out the code of foreignness HI7T213 of this invention is made to specialize, and is obtained is useful in the cell biological examination of foreignness HI7T213 in in vitro ones.

[0022]

the transgenics to an embryonic stem cell — a calcium phosphate coprecipitation Mr. method and electric punching (electroporation) — law, the RIPOFE cushion method, the retrovirus infecting method, a condensation method, and micro impregnation (microinjection) — law and a gene gun (party Kurgan) — although both law, the DEAE-dextran method, etc. can be used, generally the electroporation method is chosen from points, like many cells can be processed simple. After carrying out trypsinization of the embryonic stem cell in a logarithmic growth phase that what is necessary is just to use for electroporation the conditions currently used for the transgenics to the usual animal cell as they are and distributing a single cell, it can suspend in a culture medium and can move to a cuvette so that it may be set to 106 to 108 cell / ml, and 10–100microg addition of the vector containing DNA which carries out the code of foreignness HI7T213 can be done, and it can carry out by impressing the electric pulse of 200 – 600 V/cm.

[0023]

Although the embryonic stem cell in which the introductory gene was included can be authorized also by screening the chromosome DNA which carried out the separation extract of the single cell from the colony cultivated and obtained on a feeder cell by Southern hybridization or the PCR method, the greatest advantage of the transgenic system using an embryonic stem cell is being able to choose a transformant in a cell phase by making the manifestation of a drug resistance gene or a reporter gene into an index. Therefore, as for the introductory vector used here, it is desirable to include further selective marker genes (example: a beta-galactosidase (lacZ) gene, chloramphenicol acetyltransferase (cat) gene, etc.), such as drug resistance genes (example: a neomycin phosphotransferase II (nptII) gene, hygromycin phosphotransferase (hpt) gene, etc.) and a reporter gene, in addition to the manifestation cassette containing the gene which carries out the code of foreignness HI7T213. For example, when the vector which contains a nptII gene as a selective marker gene is used, after cultivating the embryonic stem cell after

transgenics processing in the culture medium containing neomycin system antibiotics, such as G418, moving the resistance colony which appeared to a culture plate, respectively and repeating trypsinization and culture-medium exchange, existence of an introductory gene is checked, leaving a part as an object for culture and applying the remainder to PCR or Southern hybridization.

[0024]

If the embryonic stem cell by which the nest of an introductory gene was checked is returned in the germ of the nonhuman mammal origin of the same kind, it will be included in ICM of a host germ and a chimera germ will be formed. A chimera transgenic animal is obtained by transplanting this to assumed parents (\*\*\*\*\*) and continuing generating further. When an embryonic stem cell contributes to formation of the primordial germ cell which will specialize in an egg or a sperm in the future in a chimera animal, a germ cell line chimera will be obtained and an introductory gene can create the transgenics nonhuman mammal fixed hereditarily by crossing this.

[0025]

Although there are an approach (the set chimera method) of pasting up the early embryos of a morula term and making it gathering as the production approach of a chimera germ, and the approach (the impregnation chimera method) of carrying out micro impregnation of the cell into the blastocoele of a blastocyst Although the latter is conventionally performed widely in production of the chimera germ by the embryonic stem cell The approach of making a set chimera from recently by impregnation of the embryonic stem cell into the zona pellucida of 8 cell term germ and a micromanipulator are unnecessary. Actuation as an easy approach The approach of producing a set chimera is also performed by making 8 cell term germ which removed an embryonic stem cell lump and zona pellucida cocultivate and condense.

In any case, a host germ can extract similarly from the nonhuman mammal which may be used as a female for egg gathering in the transgenics to a fertilized egg, but it is desirable to extract a host germ from the mouse of a network with which the color of hair differs from the network in which an embryonic stem cell originates in the case of a mouse, for example so that the contribution of the embryonic stem cell to chimeric-mouse formation can be judged with the color of hair (coat color). For example, if an embryonic stem cell is the 129 system mouse (color of hair: agouti) origin C57BL/6 mouse (color of hair: black) and an ICR mouse (color of hair: albino) are used as a female for egg gathering. If an embryonic stem cell is C 57B/6, or the DBF1 mouse (color of hair: black) origin and TT2 cell (F1 (color of hair: agouti) origin of C 57B/6 and CBA), an ICR mouse and a BALB/c mouse (color of hair: albino) can be used as a female for egg gathering.

Moreover, since germ cell line chimera organization potency is greatly dependent on the combination of an embryonic stem cell and a host germ, it is more desirable to choose a high combination of germ cell line chimera organization potency. For example, in the case of a mouse, it is desirable to use the host germ of C57 B/6-line origin etc. to the embryonic stem cell of the 129-line origin, and the host germ of the BALB/c network origin etc. is desirable to the embryonic stem cell of C57 B/6-line origin.

The female mouse for egg gathering has desirable about about 4-6 weeks old, and its thing of the same system of about 2 - about 8 age-of-the-moon extent is desirable as a male mouse for mating. Also by natural mating, although mating is good, after it prescribes a gonadotropic hormone (follicle-stimulating hormone, subsequently luteinizing hormone) for the patient preferably and carries out induction of the superovulation, it is performed.

[0026]

When based on the scutellum pouring-in method, a blastocyst term germ (in for example, the case of a mouse after mating about 3.5 days) is extracted from the uterus of the female for egg gathering (or after extracting the early embryo before a morula term from an oviduct). you may cultivate in the above-mentioned culture medium for embryo cultures at a blastocyst term, after pouring in the embryonic stem cell (about 10- about 15 pieces) into which DNA which carries out the code of foreignness HI7T213 into the blastocoele of a blastocyst using a micromanipulator was introduced It transplants in the uterus of the female nonhuman mammal for \*\*\*\* which carried out pseudopregnancy. The female nonhuman mammal for \*\*\*\* can use similarly the nonhuman mammal which may be used as \*\*\*\* in the transgenics to a fertilized egg.

When based on the cocultivating method, they are 8 cell term germ and morula (for example, in the case of a mouse). About 2.5 days are extracted from the oviduct and uterus of the female for egg gathering after mating (or after extracting the early embryo before 8 cell terms from an oviduct). you may cultivate in the above-mentioned culture medium for embryo cultures at 8 cell terms or a morula term, after dissolving zona pellucida in an acid Tyrode solution The embryonic stem cell lump (number of cells about 10- about 15 pieces) with which DNA which carries out the code of foreignness HI7T213 was introduced into the minute drop of the culture medium for embryo cultures which carried out multistory [ of the mineral oil ] is put in, the above-mentioned 8 cell term germ or morula (preferably two pieces) is put in further, and it cocultivates overnight. The obtained morula or the blastocyst is transplanted in the uterus of the female nonhuman mammal for \*\*\*\* like the above.

[0027]

If a transplantation germ is implanted with the sufficient result and \*\*\*\* becomes pregnant, a chimera nonhuman mammal will be obtained by the natural birth or the cesarean section. When it gives birth by the cesarean section, offspring can be made to suckle the female for breast feeding (female nonhuman mammal to which it crossed and gave birth usual) prepared separately that what is necessary is just to make \*\*\*\* which carried out the natural birth continue breast feeding as it is. Selection of a germ cell line chimera chooses the chimeric mouse of the same sex as an embryonic stem cell, when the sex of an embryonic stem cell is distinguished beforehand first (since a male embryonic stem cell is usually used, a male chimeric mouse is chosen), and subsequently it chooses a chimeric mouse (for example, 50% or more) with the high contribution of an embryonic stem cell from phenotypes, such as the color of hair. For example, in the case of the chimeric mouse obtained from the chimera germ of the D3 cell and the host germ of C57B / 6 mouse origin which are the male embryonic stem cell of the 129 system mouse origin, it is desirable to choose a male mouse with the high rate that the color of hair of an agouti occupies.



an intersection with the homozygous of a network with the appropriate check of whether the selected chimera nonhuman mammal is a germ cell line chimera -- it can carry out based on the phenotype of F1 animal obtained more coarsely. For example, the color of hair of F1 from which it will be obtained if the selected male mouse is a germ cell line chimera if it crosses with female C57B/6 mouse, since an agouti is dominant to black in the case of the above-mentioned chimeric mouse serves as an agouti.

[0028]

The germ cell line chimera nonhuman mammal (founder) into which the gene which carries out the code of the foreignness HI7T213 obtained as mentioned above was introduced is usually obtained as heterozygote which has an introductory gene only in one side of homologue. Moreover, each founder is inserted at random on a chromosome which is different unless it is based on homologous recombination. What is necessary is just to cross the brothers and sisters of heterozygote which have an introductory gene only in one side of homologue among F1 animals obtained as mentioned above, in order to obtain the homozygote which has the gene which carries out the code of foreignness HI7T213 to both homologues. Selection of heterozygote can be authorized by screening the chromosome DNA which carried out the separation extract from the tail of for example, F1 animal by Southern hybridization or the PCR method. If the introductory gene is included only in one locus, one fourth of F2 animals obtained will become a homozygote.

[0029]

As long as the amount of manifestations of foreignness HI7T213 is quantitatively secured to measurable extent in an operation of an examined substance [ as opposed to foreignness HI7T213 in the transgenic animal of this invention ], there is especially no limit about the manifestation of endogenous HI7T213. However, when using the transgenic animal of this invention also for evaluation of the drugs which can act not only on foreignness HI7T213 but on endogenous HI7T213, it is desirable to inactivate the manifestation of endogenous HI7T213. The transgenic animal of this invention by which the manifestation of endogenous HI7T213 was inactivated a well-known approach (for example, Lee S.S. et al. and Mol.Cell.Biol. --) The 15th volume, the 3012nd page, the embryonic stem cell by which HI7T213 gene chosen by reference in 1995 was knocked out, Or it can obtain by introducing the gene which carries out the code of foreignness HI7T213 to the early embryo or embryonic stem cell of the HI7T 213 knock-out animal origin created according to the above-mentioned approach from this embryonic stem cell according to the above-mentioned approach. As a concrete means to knock out HI7T213 gene HI7T213 gene of the object nonhuman mammal origin is isolated according to a conventional method. for example, the DNA fragment (for example, a neomycin resistance gene --) of others [ part / the / exon ] Drug resistance genes, such as a hygromycin tolerance gene, a lacZ gene, Or [ destroying the function of an exon by inserting reporter genes, such as a cat gene, etc. ] (in this case) The nest of an introductory gene the manifestation of drug tolerance or a reporter gene as mentioned above that it may be chosen as an index [ whether all or a part of HI7T213 genes are started using a Cre-loxP system or a Flp-frt system, and deletion of this gene is carried out, and ] Insert a termination codon into a protein coding

region, and the DNA arrays (for example, poly A addition signal etc.) which a translation of perfect protein is made [ arrays ] impossible or make the imprint of a gene end inside an imprint field are inserted. The DNA strand which has the DNA array built so that a gene might be inactivated as a result by making composition of perfect mRNA into impossible. The approach of making it build into HI7T213 locus of an object nonhuman mammal by homologous recombination (hereafter written as a targetting vector) is mentioned preferably.

[0030]

Usually, the gene recombination in mammalian has un-homonous most, and introduced DNA is inserted in the location of the arbitration of a chromosome at random. Therefore, it cannot choose efficiently only the clone by which targetting was carried out to endogenous HI7T213 gene which serves as a target by homologous recombination depending on selection of detecting the manifestation of drug tolerance or a reporter gene, but the check of the inclusion part by Southern technique or the PCR method is needed about all the selected clones. Then, if the herpes simplex virus origin thymidine kinase (HSV-tk) gene which gives for example, ganciclovir susceptibility to the outside of a field [ homologous / target sequence / of a targetting vector ] is connected, since the cell in which this vector was inserted at random has a HSV-tk gene, it cannot grow in a ganciclovir content culture medium, but since the cell in which targetting was carried out to endogenous HI7T213 locus by homologous recombination does not have a HSV-tk gene, it becomes ganciclovir resistance and it is chosen. Or if for example, a diphtheria toxin gene is connected instead of a HSV-tk gene, since the cell in which this vector was inserted at random will become extinct by this toxin that self produces, homologous recombinant can also be chosen under drugs nonexistence.

[0031]

Or the transgenic animal of this invention by which the manifestation of endogenous HI7T213 was inactivated may be a knock in animal which permuted endogenous HI7T213 gene by DNA which carries out the code of the foreignness HI7T213 gene by gene targetting which used homologous recombination.

A knock in animal is fundamentally [ as a knock out animal ] producible according to the same technique. For example, what is necessary is to excise the exon of HI7T213 gene of the object nonhuman mammal origin using a suitable restriction enzyme, to introduce into the embryonic stem cell of the object nonhuman mammal origin the targeting vector containing DNA obtained by inserting the field corresponding to instead of in foreignness HI7T213 gene according to the above-mentioned approach, and just to choose the embryonic stem cell clone in which the gene which carries out the code of foreignness HI7T213 to endogenous HI7T213 locus of this animal by homologous recombination was included. Although it can also carry out using the PCR method or Southern technique, if a clonal selection inserts marker genes for positive selection, such as a neomycin resistance gene, in 3' untranslation region of HI7T213 gene of a targetting vector etc. and marker genes for negative selection, such as a HSV-tk gene and a diphtheria toxin gene, are inserted in the outside of a field [ still homologous as a target sequence ] for example, it can make drug tolerance an index and can choose homologous recombinant.

Moreover, since the manifestation of foreignness HI7T213 into which the marker

gene for positive selection was introduced may be barred, it is desirable to start the marker gene for positive selection by making Cre, Flp recon BINAZE, or these recon BINAZE expression vectors (example: adenovirus vector etc.) act at the suitable stage after homologous recombinant selection using the targetting vector which allotted the loxP array or the frt array to the both ends of the marker gene for positive selection. Or instead of using a Cre-loxP system and a Flp-frt system, a target sequence and a homologous array may be repeated and arranged in this direction to the both ends of the marker gene for positive selection, and the marker gene for positive selection may be started using the intragenic recombination during this array.

[0032]

Moreover, the transgenic animal of this invention may be a disease model which has other one or more gene alterations which produce symptoms the same as that of the disease in which activity accommodation of HI7T213 participates, or similar.

It should be grasped as "the disease in which activity accommodation of HI7T213 participates" as a concept not only including the disease which originates in the abnormalities of HI7T213 activity, or produces the abnormalities of HI7T213 activity as a result but the disease from which prevention and/or a curative effect may be acquired by adjusting HI7T213 activity. For example, by activating HI7T213, as a disease in which prevention and/or a therapy are possible, when a wound, injury of spinal cord, or the analgesia checks HI7T213, cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, or a hyperalgesia is mentioned as a disease in which prevention and/or a therapy are possible, respectively.

Gene alteration" means the gene alteration except the gene which carries out the code of foreignness HI7T213 being introduced. "— others — The natural onset animal used in disease modeling by which the endogenous gene was changed by spontaneous mutation, The transgenic animal further introduced in other genes, the knock out animal by which inactivation was carried out in the endogenous gene (everything but the gene disruption by insertion mutation etc.) Gene expression's being undetectable or the dominant negative variant containing the transgenic animal which fell even to extent which can be disregarded into which the variation endogenous gene was introduced is contained by installation of DNA which carries out the code of an antisense DNA or the neutralizing antibody. therefore, the alteration of endogenous HI7T213 gene can also be set to this invention — "— others — it corresponds to gene alteration."

[0033]

As "a disease model which has other one or more gene alterations which produce symptoms the same as that of the disease in which activity accommodation of HI7T213 participates, or similar" as hyperlipidemia or an arteriosclerosis model — a WHHL rabbit (;Watanabe Y. which has variation in a low-density-lipoprotein receptor (LDLR) —) Atherosclerosis, the 36th volume, the 261st page, 1980, SHLM (natural onset mouse; which has apoE deficit variation — Matsushima Y. et al. —)

Mamm.Genome, the 10th volume, the 352nd page, 1999, a LDLR knockout mouse (Ishibashi S. et al. and J.Clin.Invest. —) the 92nd volume, the 883rd page, 1993, and an apoE knockout mouse (Piedrahita J.A. et al. —) Proc.Natl.Acad.Sci.USA, the 89th volume, the 4471st page, 1992, and a Homo sapiens apoB installation mouse (Callow

M.J. et al. —) Proc.Natl.Acad.Sci.USA, the 91st volume, the 2130th page, 1994, etc. — as an ischemic-heart-disease model — a CD55/CD59 double transgenic mouse (Cowan P.J. et al. —) Xenotransplantation, the 5th volume, the 184 – 90th page, 1998 etc. — as the cerebral hemorrhage or a cerebral infarction model — a CuZn-superoxide DISU mutase transgenic mouse (Saito A. et al. —) Stroke, the 2nd volume, 1652 pages, 2001, etc. — as a dermatitis model — interleukin 1 transgenic mouse (Groves R.W. et al. —) The 92nd volume of Proc.Natl.Acad.Sci.USA, 11874 pages, 1995, etc. — as an immune disorder model — CD19 knockout mouse (Spielman J. et al. —) Immunity, the 3rd volume, 39 pages, 1995, etc. — as a hypoglycemia model — SPC2 knockout mouse (Furuta M. et al. —) Proc.Natl.Acad.Sci.USA, the 94th volume, 6646 pages, 1997, etc. — as a fatty liver model — an ob/ob mouse (Herberg L. and Coleman D.L. —) Metabolism, the 26th volume, the 59th page, 1977, and KK mouse (Nakamura M. and Yamada K. —) Diabetologia, the 3rd volume, the 212nd page, 1967 pages, and a FLS mouse (Soga M. et al. —) Lab.Anim.Sci., the 49th volume, the 269th page, 1999, as a diabetes-mellitus model — a NOD mouse (Makino S. et al. and Exp.Anim. —) the 29th volume, the 1st page, 1980, and a BB rat (Crisa L. et al. —) Diabetes Metab.Rev., the 8th volume, the 4th page, 1992, an ob/ob mouse and a db/db mouse (it Science(s) Hummel L. et al. —) the 153rd volume, the 1127th page, 1966, KK mouse, and GK rat (Goto Y. et al. —) Tohoku J.Exp.Med., the 119th volume, the 85th page, 1976 and a Zucker fatty rat (Zucker L.M. et al. —) Ann.NY Acad.Sci., the 131st volume, the 447th page, 1965 and an OLETF rat (it Diabetes(es) Kawano K. et al. —) The 41st volume, the 1422nd page, 1992, etc. as an obesity model An ob/ob mouse, A db/db mouse, KK mouse, a Zucker fatty rat, An OLETF rat etc. as an Alzheimer disease model variation amyloid precursor protein trans-genic mice etc. as an anemic hypoxia model — a beta SAD transgenic mouse (beta S-Antilles-D Punjab) (Trudel M. et al. —) EMBO J, the 10th volume, 3157 pages, 1991, etc. — as a gonad failure model — Steroidogenic factor 1 knockout mouse (Zhao L. et al. —) Development, the 128th volume, 147 pages, 2001, etc. It is p53 knockout mouse (Kemp C.J.Molecular Carcinogenesis, the 12th volume) as a liver cancer model. 132 pages, 1995, etc. — as a breast cancer model — a c-neu transgenic mouse (Rao G.N. et al. —) Breast Cancer Res Treat, the 48th volume, 265 pages, 1998 etc. are mentioned for a perforin/Fas-ligand double knockout mouse (Spielman J. et al., J Immunol., the 161st volume, 7063 pages, 1998) etc. as an endometritis model. these — “— others —” disease model nonhuman mammal which has a gene alteration can be purchased from the Jackson lab in the U.S. etc., or can be easily created using a well-known gene alteration technique.

In addition to “other one or more gene alterations which produces symptoms the same as that of the disease in which activity accommodation of HI7T213 participates, or similar”, nongenetic processing which can produce the disease model of the same or others may be performed to the nonhuman mammal of this invention. “Nongenetic processing” means the processing which does not produce the gene alteration in an object nonhuman mammal. As such processing, high fat food load processing, sugar load processing, starvation processing, blood vessel ligation / reperfusion, etc. are mentioned, for example.

[0034]

To the introduced nonhuman mammal, the gene which carries out the code of foreignness HI7T213 Especially a limit does not have the approach of introducing other one or more gene alterations which produce symptoms the same as that of the disease in which activity accommodation of HI7T213 participates, or similar. For example, the nonhuman mammal introduced in the gene which carries out the code of foreignness HI7T213, Symptoms the same as that of the disease in which activity accommodation of approach; HI7T213 which cross the disease model nonhuman mammal of the same kind which has other one or more gene alterations which produce symptoms the same as that of the disease in which activity accommodation of HI7T213 participates, or similar participates, or similar To the early embryo and embryonic stem cell of a disease model nonhuman mammal which have other one or more gene alterations to produce To the early embryo and embryonic stem cell of a nonhuman mammal which were introduced, the gene which carries out the code of the approach; foreignness HI7T213 which introduce the gene which carries out the code of foreignness HI7T213 by the above-mentioned approach, and obtain a transgenic animal by the above-mentioned approach Or the approach of introducing other one or more gene alterations which produce symptoms the same as that of the disease in which activity accommodation of HI7T213 participates, or similar with a knock out technique etc. is mentioned. Moreover, when other one or more gene alterations which produce symptoms the same as that of the disease in which activity accommodation of HI7T213 participates, or similar are based on installation of a foreign gene or a dominant mutant allele, to the early embryo and embryonic stem cell of a wild type nonhuman mammal, sequential installation of the gene which carries out the code of foreignness HI7T213 to this foreign gene etc. may be carried out simultaneous, and a transgenic animal may be obtained. Furthermore, when other one or more gene alterations which produce symptoms the same as that of the disease in which activity accommodation of HI7T213 participates, or similar are based on destruction of an endogenous gene, it may design so that targetting may be carried out to the endogenous gene which should destroy the gene which carries out the code of foreignness HI7T213, and you may introduce into the embryonic stem cell of a wild type nonhuman mammal. In this case, what was illustrated about production of the above-mentioned knock in animal may be preferably used except transposing a targetting vector to the endogenous gene which should have endogenous HI7T213 gene destroyed.

[0035]

It is desirable to cross homozygotes, when crossing the disease model nonhuman mammal of the same kind which has other one or more gene alterations which produce symptoms the same as that of the nonhuman mammal introduced in the gene which carries out the code of foreignness HI7T213, and the disease in which activity accommodation of HI7T213 participates, or similar. For example, F1 from which the gene which carries out the code of foreignness HI7T213 crosses the homozygote built into one locus and an apoE gay deficit hyperlipidemia (arteriosclerosis) model, and is obtained is a hetero about both genes. It becomes foreignness HI7T 213 gay installation and an apoE gay deficit 1/16 of F2 individuals obtained by carrying out sib mating of these F1 comrades.

[0036]

although the nonhuman mammal of this invention obtained as mentioned above has agonist or antagonist activity to foreignness HI7T213 since it discovers foreignness (or it — replacing with) HI7T213 in addition to endogenous HI7T213, it makes it possible to evaluate the drug effect by in vivo one about the foreignness HI7T 213 specific agonist or the antagonist which does not have activity to endogenous HI7T213.

Moreover, when the nonhuman mammal of this invention is carrying out the superfluous manifestation of the foreignness HI7T213 gene, it has following at least one phenotype.

- (1) The symptoms of a cataract and rough hair are shown.
  - (2) A transient skin exanthema and desquamation are accepted.
  - (3) In an eyeball, fusion/denaturation of the fibrae lentis are accepted. The abnormalities of the fibrae lentis are accepted in an eyeball and stratification of the vortex lentis in the stella lentis iridica is seen in inside. That is, the symptoms of a cataract are shown.
  - (4) The view of the increment in a basophilia renal tubule is accepted with the kidney. The cell damage has occurred with the kidney. The growth promotion activity of a tubular cell is high.
  - (5) The acanthosis and parakeratosis are accepted in the skin of a juvenile period.
  - (6) The PCNA positivity cell which is a growth related antigen is accepted the stratum basale epidermidis and near pore.
  - (7) The increment in a keratin 6 gene-expression cell and the increment in a PCNA positivity cell are accepted.
- Cell proliferation promotion activity is accelerating.
- (8) The abnormalities in epidermal differentiation accompanied by manifestation sthenia of keratin 14, keratin 10, and RORIKURIN are accepted.
  - (9) In a Keratin6 positivity part, an epidermis free nerve ending is abundant.
  - (10) The manifestation of the neurotrophic factor group in the abnormality part in epidermis is reinforcing.
  - (11) The abnormalities in differentiation accompanying growth promotion activity.
- Since the nonhuman mammal of this invention has the above very unique descriptions, it has the useful application shown below.

[0037]

- (1) Since foreignness HI7T213 gene is made high-discovered, the nonhuman mammal of this invention can be used for evaluation of HI7T213 agonist or HI7T213 antagonist.

Namely, this invention,

- (i) The screening approach of HI7T213 agonist characterized by applying an examined substance to the nonhuman mammal of this invention, or its part, and authorizing HI7T 213 agonist activity or HI7T 213 antagonist activity, or HI7T213 antagonist,
- (ii) An examined substance is applied to the nonhuman mammal of this invention, or its part, and the screening approach of HI7T213 agonist used for prevention of the above-mentioned disease characterized by authorizing the improvement effect or the kidney playback effectiveness of a disease, such as a wound, injury of spinal cord, or analgesia, and/or a therapy is offered.

The candidate compound of HI7T213 agonist can be selected by joint experiment

with HI7T213.

Specifically by the screening approach of this invention, the nonhuman mammal of this invention is medicated with a specimen material. As an examined substance, the organization (for example, mouse, rat, Buta, cow, sheep, ape, Homo sapiens, etc.) extract of mammalian, cell culture supernatant liquid, etc. are used else [, such as a well-known synthetic compound, a peptide, protein, and a DNA library ].

As HI7T 213 agonist activity, an improvement operation of diseases, such as a wound, injury of spinal cord, or analgesia, or a kidney retroaction is mentioned. Fluctuation of the above-mentioned operation by HI7T213 which the nonhuman mammal of this invention has essentially may be disregarded.

After the screening approach of this invention gives a blemish to the nonhuman mammal of this invention or damages a spine, specifically, it prescribes a specimen material for the patient. And compared with the case where a specimen material is not prescribed for the patient, preferably, symptoms, such as a wound at the time of prescribing a specimen material for the patient and injury of spinal cord, can choose this specimen material about 30% or more about 10% or more as matter which has prevention and/or a curative effect to the above-mentioned disease, when improved about 50% or more more preferably.

On the other hand, as HI7T 213 antagonist activity, an improvement operation of diseases, such as cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, and a hyperalgesia, is mentioned.

Specifically, the screening approach of this invention prescribes a specimen material for the patient, after making the nonhuman mammal of this invention show the symptoms of the above-mentioned disease. And compared with the case where a specimen material is not prescribed for the patient, preferably, symptoms, such as the cancer at the time of prescribing a specimen material for the patient, renal dysfunction, a cataract, dermatitis, a chronic pain, and a hyperalgesia, can choose this specimen material about 30% or more about 10% or more as matter which has prevention and/or a curative effect to the above-mentioned disease, when improved about 50% or more more preferably.

[0038]

thus, HI7T213 selected agonist — safe — low — it can be used as prevention and/or the remedy, or kidney playback medicine of diseases, such as a toxic wound, injury of spinal cord, and analgesia.

HI7T213 antagonist chosen on the other hand — safe — low — toxic cancer and renal dysfunction, a cataract, dermatitis, a chronic pain, and nociception — it can be used as which sensitive prevention and/or a remedy.

HI7T213 agonist or HI7T213 antagonist has the desirable acid addition salt which the salt may be formed, and a salt with acids (the example, inorganic acid, etc.), bases, etc. (an example, organic acid, etc.) which are permitted physiologically is used as a salt of HI7T213 agonist or HI7T213 antagonist, and is especially permitted physiologically. As such a salt, a salt with inorganic acids (for example, a hydrochloric acid, a phosphoric acid, a hydrobromic acid, a sulfuric acid, etc.) or a salt with organic acids (for example, an acetic acid, a formic acid, a propionic acid, a fumaric acid, a maleic acid, a succinic acid, a tartaric acid, a citric acid, a malic acid, oxalic acid, a benzoic acid, methansulfonic acid, benzenesulfonic acid, etc.) is used, for example.

HI7T213 selected agonist or HI7T213 selected antagonist can be parenterally used in the form of injections, such as water, an axenic solution with the other liquid which can be permitted pharmacologically, or a suspension agent, in taking orally as the tablet and capsule which gave glycocalyx if needed, elixirs, a microcapsule agent, etc. For example, it can manufacture by mixing with the matter chosen as physis for prevention and/or a therapy with the unit dosage gestalt required of the pharmaceutical preparation implementation generally accepted with the support and the flavor agent which can be accepted physiologically, an excipient, a vehicle, antiseptics, the stabilizer, the binder, etc. A dosage with the directed range suitable for the amount of active principles in these pharmaceutical preparation is obtained. As an additive which can mix with a tablet, a capsule, etc., a flavor agent like plumping agents, such as gelatin, corn starch, tragacanth, a binder like gum arabic, an excipient like a crystalline cellulose, corn starch, gelatin, and an alginic acid, lubricant like magnesium stearate, cane sugar, a lactose or a sweetening agent like saccharin, peppermint, a dirt mono-oil, or a cherry etc. is used, for example. When dispensing unit form voice is a capsule, liquefied support still like fats and oils can be contained into said type of ingredient. The sterile constituent for injection can prescribe natural appearance vegetable oil, such as an active substance in a vehicle like water for injection, sesame oil, and coconut oil, etc. according to the usual pharmaceutical preparation implementation of making it dissolve or suspend etc.

As aquosity liquid for injection, the isotonic solutions (for example, D-sorbitol, D-mannitol, a sodium chloride, etc.) containing the adjuvant of a physiological saline, grape sugar, or others etc. are mentioned, for example, and you may use together with a suitable solubilizing agent (for example, ethanol etc.), for example, alcohol, polyalcohols (for example, propylene glycol, a polyethylene glycol, etc.), nonionic surfactants (for example, polysorbate 80 TM, HCO-50, etc.), etc. As oily liquid, sesame oil, soybean oil, etc. are mentioned and you may use together with benzyl benzoate, benzyl alcohol, etc. as a solubilizing agent, for example. Moreover, you may blend with buffers (for example, a phosphate buffer, the sodium acetate buffer solution, etc.), aponia-ized agents (for example, a benzalkonium chloride, procaine hydrochloride, etc.), stabilizers (for example, a human serum albumin, a polyethylene glycol, etc.), preservatives (for example, benzyl alcohol, a phenol, etc.), an antioxidant, etc. Suitable ampul is usually filled up with the prepared parenteral solution.

[0039]

Moreover, when the selected matter is DNA, after inserting the DNA concerned in suitable independent or vectors, such as a retrovirus vector, an adenovirus vector, and an adenovirus-associated virus vector, Homo sapiens or a homeotherm can be medicated according to a stock-in-trade. As it is, the DNA concerned is pharmaceutical-preparation-ized with support accepted physiologically, such as an adjuvant for promotion of intake, and can be prescribed for the patient with a catheter like a gene gun or a hydro gel catheter.

Thus, the pharmaceutical preparation obtained is safe, and since it is low toxicity, it can be prescribed for the patient to Homo sapiens or nonhuman homeotherms (for example, a rat, a mouse, a guinea pig, a rabbit, Tori, a sheep, Buta, a cow, a horse, a cat, a dog, an ape, etc.), for example.

About 0.1-100mg of about 1.0-50mg of about 1.0-20mg of these this matter is more



preferably prescribed [ in / generally / although the dose of the selected matter is different with the administration route for an object disease and administration etc., when administering HI7T213 agonist orally for the purpose of the therapy of a wound, for example / an adult (as the weight of 60kg) ] for the patient per day. Although the 1-time dose of the matter concerned changes with object diseases for administration etc. when prescribing a medicine for the patient parenterally For example, when an adult is medicated in the form of injections for the purpose of [ of a wound ] a therapy (as weight of 60kg), It is convenient per day to prescribe about about 0.1-10mg for the patient by injecting the affected part with about about 0.01-30mg of about about 0.1-20mg of these these matter more preferably. The amount which converted into per weight of 60kg also in other animals can be prescribed for the patient.

[0040]

(2) moreover, the phenotype or cancer which foreignness HI7T213 gene was made to high-discover the nonhuman mammal of this invention, and was described above, renal dysfunction, a cataract, dermatitis, a chronic pain, and nociception -- the symptoms of which sensitive disease may be shown

Therefore, the nonhuman mammal of this invention can be used for evaluation of the improvement medicine of the above-mentioned phenotype, the prophylactic of the above-mentioned disease, or a remedy.

That is, this invention applies an examined substance to the nonhuman mammal of this invention, or its part, and offers the screening approach of the matter used for prevention of the above-mentioned disease characterized by authorizing the improvement effect of the above-mentioned phenotype or a disease, and/or a therapy.

Specifically by the screening approach of this invention, the nonhuman mammal of this invention is medicated with a specimen material. As an examined substance, the organization (for example, mouse, rat, Buta, cow, sheep, ape, Homo sapiens, etc.) extract of mammalian, cell culture supernatant liquid, etc. are used else [ such as a well-known synthetic compound, a peptide, protein, and a DNA library ].

In the screening approach of this invention by administration of a specimen material The above-mentioned phenotype or cancer, When judged with there being an improvement effect of diseases, such as renal dysfunction, a cataract, dermatitis, a chronic pain, and a hyperalgesia (When [ for example, ] the above-mentioned phenotype or a disease has been improved about 50% or more more preferably about 30% or more about 10% or more), the specimen material can be chosen as prevention of these diseases, and/or physic for a therapy.

The matter chosen in the screening approach of this invention is for example, HI7T213 antagonist, the matter which checks the manifestation of HI7T213 (control), matter which checks HI7T213 promoter's promoter activity (control).

[0041]

The selected matter can be parenterally used in the form of injections, such as water, an axenic solution with the other liquid which can be permitted pharmacologically, or a suspension agent, in taking orally as the tablet and capsule which gave glycocalyx if needed, elixirs, a microcapsule agent, etc. For example, it can manufacture by mixing with the matter chosen as physic for prevention and/or a

therapy with the unit dosage gestalt required of the pharmaceutical preparation implementation generally accepted with the support and the flavor agent which can be accepted physiologically, an excipient, a vehicle, antiseptics, the stabilizer, the binder, etc. A dosage with the directed range suitable for the amount of active principles in these pharmaceutical preparation is obtained.

As an additive which can mix with a tablet, a capsule, etc., a flavor agent like plumping agents, such as gelatin, corn starch, tragacanth, a binder like gum arabic, an excipient like a crystalline cellulose, corn starch, gelatin, and an alginic acid, lubricant like magnesium stearate, cane sugar, a lactose or a sweetening agent like saccharin, peppermint, a dirt mono-oil, or a cherry etc. is used, for example. When dispensing unit form voice is a capsule, liquefied support still like fats and oils can be contained into said type of ingredient. The sterile constituent for injection can prescribe natural appearance vegetable oil, such as an active substance in a vehicle like water for injection, sesame oil, and coconut oil, etc. according to the usual pharmaceutical preparation implementation of making it dissolve or suspend etc.

As aqueous liquid for injection, the isotonic solutions (for example, D-sorbitol, D-mannitol, a sodium chloride, etc.) containing the adjuvant of a physiological saline, grape sugar, or others etc. are mentioned, for example, and you may use together with a suitable solubilizing agent (for example, ethanol etc.), for example, alcohol, polyalcohols (for example, propylene glycol, a polyethylene glycol, etc.), nonionic surfactants (for example, polysorbate 80 TM, HCO-50, etc.), etc. As oily liquid, sesame oil, soybean oil, etc. are mentioned and you may use together with benzyl benzoate, benzyl alcohol, etc. as a solubilizing agent, for example. Moreover, you may blend with buffers (for example, a phosphate buffer, the sodium acetate buffer solution, etc.), aponia-ized agents (for example, a benzalkonium chloride, procaine hydrochloride, etc.), stabilizers (for example, a human serum albumin, a polyethylene glycol, etc.), preservatives (for example, benzyl alcohol, a phenol, etc.), an antioxidant, etc. Suitable ampul is usually filled up with the prepared parenteral solution.

[0042]

Moreover, when the selected matter is DNA, after inserting the DNA concerned in suitable independent or vectors, such as a retrovirus vector, an adenovirus vector, and an adenovirus-associated virus vector, Homo sapiens or a homeotherm can be medicated according to a stock-in-trade. As it is, the DNA concerned is pharmaceutical-preparation-ized with support accepted physiologically, such as an adjuvant for promotion of intake, and can be prescribed for the patient with a catheter like a gene gun or a hydro gel catheter.

Thus, the pharmaceutical preparation obtained is safe, and since it is low toxicity, it can be prescribed for the patient to Homo sapiens or nonhuman homeotherms (for example, a rat, a mouse, a guinea pig, a rabbit, Tori, a sheep, Buta, a cow, a horse, a cat, a dog, an ape, etc.), for example.

About 0.1-100mg of about 1.0-50mg of about 1.0-20mg of these this matter is more preferably prescribed [ in / generally / although the dose of the selected matter is different with the administration root for an object disease and administration etc., when administering orally for the purpose of the therapy of a cataract, for example / an adult (as the weight of 60kg) ] for the patient per day. Although the 1-time dose of the matter concerned changes with object diseases for administration etc. when

prescribing a medicine for the patient parenterally For example, when an adult is medicated in the form of injections for the purpose of [ of a cataract ] a therapy (as weight of 60kg), It is convenient per day to prescribe about about 0.1–10mg for the patient by injecting the affected part with about about 0.01–30mg of about about 0.1–20mg of these these matter more preferably. The amount which converted into per weight of 60kg also in other animals can be prescribed for the patient. Furthermore, the nonhuman mammal of this invention can be used for the experiment for gene therapies of a HI7T 213 abnormality-of-the-genes patient.

[0043]

It is also possible to use the transgenics mammalian of the above this invention as a source of a cell for tissue culture. Moreover, it is also analyzable about relevance with the transcription factor of a complicated operation of the receptor in a nucleus by analyzing the protein organization which did the direct method of analysis of DNA or RNA under organization of the trans-genic mice of this invention, or was discovered by the gene, for example. Or the function of a cell in which culture originates in a difficult organization generally [ the cell which cultivates the cell of the organization which has a gene with a normal structure culture technique, and uses these, for example, forms fat tissue ] can also be studied. Furthermore, selection of drugs which raise the function of a cell is also possible by using the cell. Moreover, if there is a high manifestation cell strain, it is also possible to carry out isolation purification of HI7T213 in large quantities from there and to produce the antibody.

[0044]

The G-protein conjugation mold receptor protein (it may be hereafter written as HI7T213) used by this invention is receptor protein which contains the same amino acid sequence identically to the amino acid sequence expressed with array number:1, array number:3, array number:5, or array number:7, or substantially.

HI7T213 — for example, Homo sapiens and a nonhuman mammal (for example, a guinea pig —) All cells, such as a rat, a mouse, a rabbit, Buta, a sheep, a cow, and an ape for example, splenic cells, a nerve cell, a neuroglia, a pancreas beta cell, and a bone marrow cell — A mesangial cell, Langerhans cell, an epidermal cell, an epithelial cell, an endothelial cell, fibrocyte, a desmacyte, a muscle cell, a fat cell, and immunocyte (an example and a macrophage —) A T cell, a B cell, a spontaneous killer cell, a mast cell, neutrophil leucocyte, basophilic leucocyte, Eosinophile leucocyte, monocyte, megakaryocyte, a synovial cell, chondrocyte, osteocyte, osteoblast, An osteoclast, an alveolar epithelial cell, hepatocyte, an interstitial cell, or the precursor cell of these cells, All the organizations where a stem cell or a gun cell, the cells of a corpuscle system, or those cells exist, about (an example, an olfactory bulb, an amygdala, and a cerebrum — the base — a ball, a hippocampus, and a thalamus —) each part of a brain and a brain Hypothalamus, a subthalamic nucleus, the cerebral cortex, a medulla oblongata, a cerebellum, an occipital lobe, the frontal lobe, a temporal lobe, Putamen, a caudate nucleus, \*\*\*\*, substantia nigra, a spine, a hypophysis, the stomach, the pancreas, the kidney, liver, a gonad, the thyroid, the gallbladder, bone marrow, a suprarenal gland, the skin, muscles, lungs, and an alimentary canal (an example —) You may be protein originating in the large intestine, a small intestine, a blood vessel, the heart, a thymus gland, a spleen, an submaxillary

gland, peripheral blood, a peripheral blood ball, a prostate gland, a testis, a testis, the ovary, a placenta, a uterus, a bone, a joint, skeletal muscle, etc., and may be synthetic protein.

[0045]

Array number: The amino acid sequence which has about 95% or more of homology still more preferably is mentioned about 90% or more more preferably 80% or more as substantially as the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7 preferably about 76% or more as the amino acid sequence expressed with array number:1, array number:3, array number:5, or array number:7 as the same amino acid sequence, for example.

The array number of this invention : as the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, and protein which contains the same amino acid sequence substantially For example, \*\*\*\*\* array number:1, array number:3, array number:5, or an array number : It has the same amino acid sequence substantially with the amino acid sequence expressed with 7. Array number: The amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, the protein which has homogeneous activity substantially are desirable.

The homology of an amino acid sequence is homology computational algorithm NCBI. It is calculable on condition that the following (expected-value = 10;; which allows a gap matrix = BLOSUM62; filtering = OFF) using BLAST (National Center for Biotechnology Information Basic Local Alignment Search Tool).

As homogeneous activity, ligand avidity, a signal signal transduction operation, etc. are mentioned substantially, for example. It is substantially indicated to be the same quality that those activity is homogeneous in property. Therefore, although it is desirable that activity, such as ligand avidity and a signal signal transduction operation, is EQCs (an example, about 0.01 to 100 times, preferably about 0.5 to 20 times, more preferably about 0.5 to 2 twice), quantitative elements, such as extent of such activity and proteinic molecular weight, may differ.

Measurement of activity, such as ligand avidity and a signal signal transduction operation, can be measured according to the screening approach indicated later, for example, although it can carry out according to a well-known approach.

[0046]

Moreover, as HI7T213, it is 1 in the amino acid sequence expressed with a array number:1, array number:3, array number:5, or array number:7, or two pieces or more (preferably). The amino acid sequence in which about 1-30 amino acid [ about 1-10 / some (1-5 pieces) still more preferably ] carried out deletion more preferably, b) -- array number: -- the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7 -- 1 or two pieces or more (preferably) The amino acid sequence which about 1-30 amino acid [ about 1-10 / some (1-5 pieces) still more preferably ] added more preferably, c) -- array number: -- 1 in the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7, or two pieces or more (preferably) the amino acid sequence by which about 1-30 amino acid [ about 1-10 / some (1-5 pieces) still more preferably ] was more preferably permuted from other amino acid, or d) -- the protein containing the amino acid sequence which combined them etc. is used.

## [0047]

According to the practice of a peptide mark, a left end is an amino terminal (amino terminus), and the right end of HI7T213 in this specification is a C terminal (carboxyl terminus). Array number: The C terminals of HI7T213 including HI7T213 containing the amino acid sequence expressed with 1 may be any of a carboxyl group ( $-\text{COOH}$ ), carboxylate ( $-\text{COO}^-$ ), an amide ( $-\text{CONH}_2$ ), or ester ( $-\text{COOR}$ ).

As R in ester, here, for example Methyl, ethyl, n-propyl, C1-6 alkyl groups, such as isopropyl or n-butyl For example, C3-8 cycloalkyl radicals, such as cyclopentyl and cyclohexyl, For example, C6-12 aryl groups, such as phenyl and alpha-naphthyl For example, the pivaloyloxymethyl radical used widely as ester for taking orally besides C7-14 aralkyl radicals, such as alpha-naphthyl-C1-2 alkyl groups, such as phenyl-C1-2 alkyl groups, such as benzyl and phenethyl, or alpha-naphthyl methyl, is used.

When HI7T213 have the carboxyl group (or carboxylate) in addition to the C terminal, that by which the carboxyl group is amidated or esterified is also contained in HI7T213 of this invention. As ester in this case, the ester of a C terminal described above, for example is used.

In the protein described above to HI7T213 the amino group of the methionine residue of an amino terminal Furthermore, a protective group What (for example, is protected by C1-6 acyl groups, such as C2-6 alkanoyl radicals, such as a formyl group and acetyl, etc.), What the glutamyl radical which N one end was cut in the living body, and was generated pyroglutamic-acid-ized, the substituent on the side chain of the amino acid of intramolecular (for example,  $-\text{OH}$ ,  $-\text{SH}$ , and the amino group  $--$ ) A protective group with suitable imidazole group, Indore radical, guanidino radical, etc. Conjugated protein, such as a thing (for example, protected by C1-6 acyl groups, such as C2-6 alkanoyl radicals, such as a formyl group and acetyl, etc.) or the so-called glycoprotein which the sugar chain combined, etc. is contained.

Homo-sapiens origin HI7T213 which consist of an amino acid sequence expressed with array number:1 as an example of HI7T213 of this invention, for example, Homo-sapiens origin HI7T213 which consist of an amino acid sequence expressed with array number:3, mouse origin HI7T213 which consist of an amino acid sequence expressed with array number:5, an array number: Mouse origin HI7T213 which consist of an amino acid sequence expressed with 7 are used. Among these, an array number: Mouse origin HI7T213 which consist of an amino acid sequence expressed with 5 are new receptor protein.

## [0048]

Although you may be which thing as long as it is the above-mentioned partial peptide of HI7T213 as a partial peptide (it may be hereafter written as a partial peptide) of HI7T213, it is the part exposed out of a cell membrane among the protein molecules of HI7T213, and what has homogeneous receptor avidity substantially is used, for example.

It is a peptide containing the part analyzed to be an extracellular field (hydrophilic (Hydrophilic) part) in hydrophobic plot analysis as a partial peptide of HI7T213 which specifically have the amino acid sequence expressed with array number:1, array number:3, array number:5, or array number:7. Moreover, the peptide which includes a hydrophobic (Hydrophobic) part in a part can be used similarly. Although the peptide

which includes each domain according to an individual can also be used, the peptide of the part which includes two or more domains in coincidence is sufficient. The number of the amino acid of the partial peptide of this invention has [ at least 20 or more in the configuration amino acid sequence of the receptor protein of above-mentioned this invention ] the preferably desirable peptide which has 100 or more amino acid sequences more preferably 50 or more pieces. The same amino acid sequence shows substantially the amino acid sequence which has about 95% or more of homology still more preferably about 90% or more more preferably about 80% or more as preferably about 76% or more as these amino acid sequences.

The homology of an amino acid sequence is the same homology computational algorithm NCBI as the above. It is calculable on the same conditions using BLAST. Here, it is indicated the above and this meaning "are homogeneous receptor activity substantially." Measurement of "being homogeneous receptor activity substantially" can be performed like the above.

[0049]

Moreover, 1 in the above-mentioned amino acid sequence or two or more (preferably about 1-10 pieces, still more preferably partly (1-5 pieces)) amino acid carry out deletion of the partial peptide of this invention. Or it is 1 or two pieces or more (preferably) to the amino acid sequence. About 1-20 amino acid [ some (1-5 pieces) still more preferably ] adds about 1-10 pieces more preferably. Or 1 in the amino acid sequence or two or more (preferably about 1-10 pieces, more preferably partly still more preferably about 1-5 pieces) amino acid may be permuted by other amino acid.

Moreover, the C terminals of the partial peptide of this invention may be any of a carboxyl group ( $-\text{COOH}$ ), carboxylate ( $-\text{COO}^-$ ), an amide ( $-\text{CONH}_2$ ), or ester ( $-\text{COOR}$ ). When the partial peptide of this invention has the carboxyl group (or carboxylate) in addition to the C terminal, that by which the carboxyl group is amidated or esterified is also contained in the partial peptide of this invention. As ester in this case, the ester of a C terminal described above, for example is used. Furthermore, compound peptides, such as that from which the amino group of the methionine residue of an amino terminal is protected by the protective group, a thing which the glutamyl radical which N one end was cut in the living body, and was generated pyroglutamic-acid-ized, a thing protected by the protective group with the suitable substituent on the side chain of the amino acid of intramolecular, or the so-called glycopeptide which the sugar chain combined, etc. are contained like HI7T213 described above to the partial peptide of this invention.

The acid addition salt which the salt with an acid or a base permitted physiologically is mentioned, and is especially physiologically permitted as a salt of the HI7T213 or the partial peptide of those of this invention is desirable. As such a salt, a salt with an inorganic acid (for example, a hydrochloric acid, a phosphoric acid, a hydrobromic acid, a sulfuric acid) or a salt with an organic acid (for example, an acetic acid, a formic acid, a propionic acid, a fumaric acid, a maleic acid, a succinic acid, a tartaric acid, a citric acid, a malic acid, oxalic acid, a benzoic acid, methansulfonic acid, benzenesulfonic acid) is used, for example.

[0050]

HI7T213 or the salt of those of this invention can also be manufactured by the purification approach of well-known receptor protein from the cell or organization of above-mentioned Homo sapiens and mammalian, and can be manufactured also by cultivating the transformant containing DNA which carries out the code of HI7T213 of this invention indicated later. Moreover, it can also manufacture according to the protein synthesis method or this which is indicated later.

When manufacturing from the tissue or the cell of Homo sapiens or mammalian, after homogenizing the tissue or the cell of Homo sapiens or mammalian, an acid etc. can extract and purification isolation of the obtained extract can be carried out by combining chromatographies, such as reversed phase chromatography and ion exchange chromatography.

[0051]

The commercial resin for protein synthesis can usually be used for composition of HI7T213 or the partial peptide of those of this invention, its salt, or its amide object. As such resin, chloro methyl resin, hydroxymethyl resin, benzhydryl amineresin, aminomethyl resin, 4-benzyloxy benzyl alcohol resin, 4-methyl benzhydryl amineresin, Pulse-Amplitude-Modulation resin, 4-hydroxymethyl methylphenyl acetamide methyl resin, polyacrylamide resin, 4-(2', 4'-dimethoxy phenyl-hydroxymethyl) phenoxy resin, 4-(2', 4'-dimethoxy phenyl-Fmoc aminoethyl) phenoxy resin, etc. can be mentioned, for example. According to the various well-known condensation approaches, condensation of the amino acid which protected alpha-amino group and a side-chain functional group suitably is carried out as the array of the protein made into the purpose on resin using such resin. Various protective groups are removed at the same time it cuts down protein from resin at the last of a reaction, an intramolecular disulfide bond formation reaction is further carried out in high diluted solution, and target protein or its target amide object is acquired.

Although the various activation reagents which can be used for protein synthesis can be used about the condensation of the above-mentioned protection amino acid, carbodiimides are good especially. As carbodiimides, a DCC, N, and N'- diisopropyl carbodiimide and N-ethyl-N'-(3-dimethylamino proyl) carbodiimide etc. is used. After adding protection amino acid with a racemization control additive (for example, HOBt, HOObt) for activation by these at direct resin or activating protection amino acid beforehand as a symmetry acid anhydride, HOBt ester, or HOObt ester, it can add to resin.

[0052]

It is suitably chosen from the solvent with which it is known as a solvent used for activation of protection amino acid, or condensation with resin that it can be used for a protein condensation reaction, and gets. For example, ester or such proper mixture, such as nitril, such as ether, such as sulfoxides, such as alcohols, such as halogenated hydrocarbon, such as acid amides, such as N,N-dimethylformamide, N,N-dimethylacetamide, and N-methyl pyrrolidone, a methylene chloride, and chloroform, and trifluoro ethanol, and dimethyl sulfoxide, a pyridine, dioxane, and a tetrahydrofuran, an acetonitrile, and propionitrile, methyl acetate, and ethyl acetate, etc. are used. It is suitably chosen from the range where it is known that it may be used for a protein bonding reaction, and reaction temperature is [ about ] usually. -It is suitably chosen from the range of 20 degrees C - 50 degrees C. 1.5 to 4 times of

the activated amino acid derivative are usually superfluous, and it is used. Sufficient condensation can be performed by repeating a condensation reaction as a result of the test using ninhydrin reaction, without performing desorption of a protective group, when condensation is inadequate. Even if it repeats a reaction, when sufficient condensation is not obtained, unreacted amino acid can be acetylated using an acetic anhydride or acetylimidazole.

[0053]

As a protective group of the amino group of a raw material, Z, Boc, tertiary pentyloxy carbonyl, isobornyl oxy-carbonyl, 4-methoxybenzyloxy carbonyl, Cl-Z, Br-Z, adamantyloxy carbonyl, trifluoro acetyl, phthloyl, the formyl, 2-nitrophenylsulfenyl, diphenylphosphinothiyl, Fmoc, etc. are used, for example.

a carboxyl group -- for example, alkyl esterification (for example, methyl --) Ethyl, propyl, butyl, tertiary butyl, cyclopentyl, The shape of a straight chain, such as cyclohexyl, cycloheptyl one, cyclo octyl, and 2-adamantyl, The shape of branching, annular alkyl esterification, aralkyl esterification for example, benzyl ester, 4-nitrobenzyl ester, and 4-methoxybenzyl ester -- It can protect by 4-chloro benzyl ester, benzhydryl esterification, phenacyl esterification, the formation of benzyloxycarbonyl hydrazide, the formation of tertiary butoxycarbonyl hydrazide, trityl hydrazide-ization, etc.

The hydroxyl group of a serine can be protected according to esterification or etherification. As a radical suitable for this esterification, the radical guided from carbonic acid, such as aroyl radicals, such as low-grade alkanoyl radicals, such as an acetyl group, and benzoyl, a benzyloxycarbonyl radical, and an ethoxycarbonyl radical, is used, for example. Moreover, as a radical suitable for etherification, they are benzyl, a tetrahydropyranyl group, t-butyl, etc., for example.

As a protective group of the phenolic hydroxyl group of a tyrosin, Bzl, Cl<sub>2</sub>-Bzl, 2-nitrobenzyl, Br-Z, tertiary butyl, etc. are used, for example.

As a protective group of the imidazole of a histidine, they are Tos and 4-methoxy, for example. - 2, 3, 6-trimethyl benzenesulphonyl, DNP, benzyloxymethyl, Bum, Boc and Trt, Fmoc, etc. are used.

[0054]

As that by which the carboxyl group of a raw material was activated, a corresponding acid anhydride, azide, activity ester [ester with alcohol (for example, pentachlorophenol, 2 and 4, 5-trichlorophenol, a 2, 4-dinitrophenol, cyano methyl alcohol, a PARANITORO phenol, HONB, N-hydroxy SUKUSHIMIDO, an N-hydroxy phthalimide, HOBt)], etc. are used, for example. As that by which the amino group of a raw material was activated, a corresponding phosphoric-acid amide is used, for example.

as the removal (desorption) approach of a protective group -- the catalytic reduction in the inside of the hydrogen air current under existence of the catalyst of for example, Pd-black or Pd-carbon -- moreover, the base processing by the acid treatment by anhydrous hydrogen fluoride, methansulfonic acid, trifluoro methansulfonic acid, trifluoroacetic acid, or these mixed liquor, diisopropyl ethylamine, triethylamine, a piperidine, a piperazine, etc., reduction by the sodium in liquid ammonia, etc. are used. Generally the elimination reaction by the above-mentioned acid treatment is [ about ]. -Although carried out at the



temperature of 20 degrees C – 40 degrees C, in acid treatment, addition of cation scavengers, such as anisole, phenol, thioanisole, metacresol, Parakou resol, dimethyl sulfide, 1, 4-butane dithiol, 1, and 2-ethane dithiol, is effective, for example. Moreover, the 2,4-dinitrophenyl radical used as an imidazole protective group of a histidine is removed by thiophenol processing, and the formyl group used as an Indore protective group of a tryptophan is removed by the alkali treatment by the rare sodium-hydroxide solution, rare ammonia, etc. in addition to the deprotection by the acid treatment under existence of above 1, 2-ethane dithiol, 1, and 4-butane dithiol etc.

[0055]

The protection and the protective group of the functional group which does not come out which should participate in the reaction of a raw material and desorption of the protective group, activation of the functional group which participates in a reaction, etc. can be suitably chosen from a well-known radical or a well-known means.

As an option which acquires a proteinic amide object For example, after amidating and protecting alpha-carboxyl group of carboxy end amino acid first, After even the chain length of a request of a peptide (protein) chain extends to an amino-group side, The protein only except the protective group of alpha-amino group of the amino terminal of this peptide chain and the protein from which only the protective group of the carboxyl group of a C terminal was removed are manufactured, and condensation is carried out in a mixed solvent which described both this protein above. About the detail of a condensation reaction, it is the same as that of the above. After refining the protection protein obtained by condensation, all protective groups can be removed by the above-mentioned approach, and desired crude protein can be obtained. This crude protein can be refined making full use of various known purification means, and the amide object of desired protein can be acquired by freeze-drying main fractions.

After condensing with the alcohols of a request of alpha-carboxyl group of carboxy end amino acid and considering as amino acid ester in order to acquire a proteinic ester object for example, the ester object of desired protein as well as a proteinic amide object can be acquired.

[0056]

The partial peptide of HI7T213 of this invention or its salt can be manufactured by cutting HI7T213 of this invention by the suitable peptidase according to the synthesis method of a well-known peptide. As a synthesis method of a peptide, it is good in both a solid phase synthesis method and a liquid phase synthesis method, for example. That is, condensation of the partial peptide or amino acid which can constitute HI7T213 of this invention, and the residual part is carried out, and when a product has a protective group, the target peptide can be manufactured by being desorbed from a protective group. As desorption of the well-known condensation approach or a protective group, the approach indicated by the following a-e, for example is mentioned.

a) M.Bodanszky It reaches. M.A.Ondetti, peptide Synthesis (Peptide Synthesis) Interscience Publishers and New York (1966)

b) Schroeder and Luebke, THE A peptide (The Peptide), Academic Press, and New York (1965)

c) The foundation of peptide synthesis besides Izumi store Nobuo, and experiment, Maruzen Co., Ltd. (1975)

d) Haruaki Yajima And Shunpei Sakakibara, a biochemistry experiment lecture 1, The proteinic chemistry IV and 205 (1977),

e) Haruaki Yajima editorial supervision, development of \*\*\*\*\* The 14th volume Peptide synthesis Hirokawa bookstore

Moreover, after a reaction can carry out purification isolation of the partial peptide of this invention combining the usual purification method, for example, solvent extraction, distillation, and a column chromatography, liquid chromatography, recrystallization, etc. It is convertible for a suitable salt by the approach that it is well-known when the partial peptide obtained by the above-mentioned approach is educt, and when conversely obtained with a salt, it can change into educt by the well-known approach.

[0057]

As long as it contains the base sequence (DNA or RNA, preferably DNA) which carries out the code of HI7T213 of above-mentioned this invention as a polynucleotide which carries out the code of HI7T213 of this invention, you may be what kind of thing. As this polynucleotide, it may be RNA which carries out the code of HI7T213 of this invention, such as DNA and mRNA, and may be a double strand, or you may be a single strand. In the case of a double strand, the hybrid of double stranded DNA, double stranded RNA, or DNA:RNA is sufficient. In the case of a single strand, it may be a sense chain (namely, code chain), or it may be an antisense strand (namely, non-code chain).

The quantum of the mRNA of HI7T213 of this invention can be carried out by the approach according to the approach of well-known experimental-medicine special number "new PCR and its application" 15(7) and 1997 publications, or it, using the polynucleotide which carries out the code of HI7T213 of this invention.

As DNA which carries out the code of HI7T213 of this invention, cDNA of genomic DNA, a genomic DNA library, the above-mentioned cell, or the organization origin, the above-mentioned cell or the cDNA library of the organization origin, and any of a synthetic DNA are sufficient. The vectors used for a library may be any, such as a bacteriophage, a plasmid, cosmid, and phagemid. Moreover, it can also amplify using what prepared totalRNA or a mRNA fraction by direct Reverse Transcriptase Polymerase Chain Reaction (it is hereafter called RT-PCR method for short) from the above-mentioned cell or the above-mentioned organization.

As DNA which carries out the code of HI7T213 of this invention, specifically For example, array number:2, array number:4, array number:6, or an array number : DNA containing the base sequence expressed with 8, It has the base sequence hybridized under stringent conditions. or array number: --- the base sequence expressed with 2, array number:4, array number:6, or array number:8, and a high --- array number: --- HI7T213 which consist of an amino acid sequence expressed with 1, array number:3, array number:5, or array number:7 --- substantial --- homogeneous activity (an example ---) Which thing may be used as long as it is DNA which carries out the code of the receptor protein which has ligand avidity, a signal signal transduction operation, etc.

Array number: DNA containing the base sequence which has about 95% or more of

homology more preferably about 90% or more etc. is preferably used about 80% or more with the base sequence expressed with 2, array number:4, array number:6, or array number:8, and the base sequence expressed with array number:2, array number:4, array number:6, or array number:8 as DNA which can be hybridized, respectively, for example.

[0058]

The homology of a base sequence is homology computational algorithm NCBI. It is calculable on condition that the following (expected-value = 10;; which allows a gap filtering = ON; match score = 1; mismatch score = -3) using BLAST (National Center for Biotechnology Information Basic Local Alignment Search Tool).

Hybridization can be performed according to the approach according to a well-known approach or well-known it, for example, an approach given in Molecular Cloning 2nd (J.Sambrook et al., Cold Spring Harbor Lab.Press, 1989) etc. Moreover, when using a commercial library, according to the approach of a publication, it can carry out to attached directions for use. more -- desirable -- a high -- it can carry out according to stringent conditions.

this high -- for example, sodium concentration is about 19 to 20 mM preferably about 19 to 40 mM, and, as for stringent conditions, temperature shows preferably about 50-70 degrees C of about 60-65-degree C conditions. Especially, the case where sodium concentration is [ temperature ] about 65 degrees C in about 19 mM(s) is the most desirable.

DNA which consists of a base sequence expressed with array number:2 as DNA which carries out the code of the Homo sapiens origin HI7T213 (hHI7T213) which more specifically consist of an amino acid sequence expressed with array number:1 is used. Array number: DNA which consists of a base sequence expressed with array number:4 as DNA which carries out the code of the Homo sapiens origin HI7T213 (hHI7T213) which consist of an amino acid sequence expressed with 3 is used. Array number: DNA which consists of a base sequence expressed with array number:6 as DNA which carries out the code of the mouse origin HI7T213 (#11) which consist of an amino acid sequence expressed with 5 is used. Array number: DNA which consists of a base sequence expressed with array number:8 as DNA which carries out the code of the mouse origin HI7T213 (#8) which consist of an amino acid sequence expressed with 7 is used.

The polynucleotide which comes to contain a part of a part of base sequence of DNA which carries out the code of HI7T213 of this invention, or this DNA and complementary base sequence not only includes DNA which carries out the code of the partial peptide of following this invention, but is used in the semantics which also includes RNA.

If this invention is followed, the antisense polynucleotide (nucleic acid) which can check the duplicate or manifestation of a GPR gene was cloned, or HI7T213 determined are designed based on the base sequence information on DNA which carries out a code, and can be compounded. Such a polynucleotide (nucleic acid) can be hybridized with RNA of HI7T213 gene, it can check composition or the function of this RNA, or can mind an interaction with the HI7T213 relation RNA, and can adjust and control HI7T213 gene expression. The polynucleotide complementary in the array as which the GPR relation RNA was chosen, and the GPR relation RNA and the

polynucleotide which can be hybridized specifically are useful to which sick therapy or sick diagnosis useful although HI7T213 gene expression is adjusted and controlled out of [ in-the-living-body and ] a living body. Although a 5' edge hairpin loop and 5' edge 6-base pair repeat, 5' edge untranslation region, polypeptide translation initiation codon, protein coding region, ORF translation initiation codon, and 3' edge untranslation region, and 3' edge palindrome field and 3' edge hairpin loop of HI7T213 gene can be chosen as a desirable object domain, any fields in HI7T213 gene can be chosen as an object of an antisense polynucleotide.

[0059]

It can be said that the relation between the purpose nucleic acid and the polynucleotide which it is complementary to a part of object domain [ at least ], and can be hybridized to it is an object and antisense [ "antisense one" ]. The polydeoxyribonucleotide in which the antisense polynucleotide contains the 2-deoxy-D-ribose, The polynucleotide of the type of others which are the polyribonucleotide containing D-ribose, a pudding, or N-glycoside of a pyrimidine base, or the polymer (for example, commercial protein —) of others which have a non-nucleotide frame a nucleic acid and a synthetic array — the polymer (however, this polymer contains a nucleotide with the arrangement which permits pairing of a base and adhesion of a base which are found out in DNA or RNA) of others containing a specific nucleic-acid polymer or special association etc. is mentioned. They The double stranded DNA, a single stranded DNA, the 2 chain RNA, single stranded RNA, Furthermore, it can be a DNA:RNA hybrid. Further A non-modified polynucleotide (or non-modified oligonucleotide), That to which still better known qualification was added, for example, a thing with the indicator known for the field concerned, that to which the cap was attached, the methylated thing, and one or more natural nucleotides — a relative — what was permuted by the object — That to which intramolecular nucleotide qualification was carried out, for example, non-electric charge association A phospho RUAMI date for example, methyl phosphonate and phospho triester — A thing with a carbamate etc., association which has a charge, or sulfur content association What (for example, has phosphorothioate, phosphorodithioate, etc.), for example, protein (nuclease, nuclease inhibitor, and toxin —) What has side chain radicals, such as an antibody, transit peptide, poly-L-lysine, etc. and sugar (for example, mono-saccharide etc.), A thing with intercalation compounds (for example, an acridine, psoralen, etc.), You may have the thing containing chelate compounds (for example, a metal, a metal with radioactivity, boron, the metal of an oxidizing quality, etc.), a thing containing an alkylating agent, and embellished association (for example, nucleic acid of alpha anomer mold etc.). It not only contains a pudding and a pyrimidine base, but what has the heterocycle mold base of others which were embellished may be included with the "nucleoside", the "nucleotide", and the "nucleic acid" here. Such a qualification object may include the heterocycle of the methylated pudding and a pyrimidine, the acylated pudding and a pyrimidine, or others. A part for a sugar part may be embellished again, for example, one or more hydroxyl groups may be permuted by the halogen, the aliphatic series radical, etc., or the embellished nucleotide and the embellished nucleotide may be changed into functional groups, such as the ether and an amine.

[0060]

The antisense polynucleotide (nucleic acid) of this invention is RNA, DNA, or the embellished nucleic acid (RNA, DNA). Although the thing of resistance is mentioned to decomposition of the sulfur derivative, thio phosphate derivative and the poly nucleoside amide of a nucleic acid, or an oligo nucleoside amide as an example of the embellished nucleic acid, it is not limited to it. The antisense nucleic acid of this invention is designed preferably, and is sold at the following plans. That is, supposing toxicity makes bigger compatibility over the sense chain which makes intracellular antisense nucleic acid more stable, which raises the cell permeability of antisense nucleic acid more and which is made into a target, the toxicity of antisense nucleic acid will be made into a smaller thing.

in this way, many qualification is got to know in the field concerned — having —

\*\*\*\* — for example, — J.Kawakami et al.,

Pharm Tech Japan, Vol.8, pp.247, and 1992; Vol.8, pp.395, and 1992; S.T.Crooke et al.ed., Antisense Research and Applications, CRC Press, and 1993 etc. — there is an indication.

The antisense nucleic acid of this invention is made to change, or may contain the embellished sugar, a base, and association, and a grant is made with liposome and a special gestalt like a microsphere, it is applied by gene therapy, or it may be given with the added gestalt. In this way, an interaction with a poly cation object like the poly lysine which works as what is used with an addition gestalt so that the charge of a phosphoric-acid radical frame may be neutralized, and a cell membrane is raised, or a thing of rough aquosity called lipids (for example, phospholipid, cholesterol, etc.) which make the incorporation of a nucleic acid increase is mentioned. As a desirable lipid, cholesterol and its derivatives (for example, cholesteryl chloro formate, cholic acid, etc.) are mentioned for adding. Such a thing can be made to adhere to 3' edge or 5' edge of a nucleic acid, and may be made to adhere through a base, sugar, and intramolecular nucleoside association. As other radicals, it is the radical for a cap arranged specifically, and the thing for preventing decomposition by nucleases, such as exonuclease and RNase, is mentioned to 3' edge or 5' edge of a nucleic acid. Although the protective group of the hydroxyl group known for the fields concerned including glycols, such as a polyethylene glycol and tetraethylene glycol, is mentioned as a radical for such a cap, it is not limited to it.

The inhibition activity of antisense nucleic acid can be investigated using the gene expression system besides the transformant of this invention, and in the living body [ of this invention ] and a living body, or the translation system besides in the living body [ of G-protein conjugation mold receptor protein ], or a living body. This nucleic acid is applicable to a cell by various kinds of well-known approaches.

[0061]

As long as it contains the base sequence which carries out the code of the partial peptide of above-mentioned this invention as DNA which carries out the code of the partial peptide of this invention, you may be what kind of thing. Moreover, cDNA of genomic DNA, a genomic DNA library, the above-mentioned cell, or the organization origin, the above-mentioned cell or the cDNA library of the organization origin, and any of a synthetic DNA are sufficient. The vectors used for a library may be any, such as a bacteriophage, a plasmid, cosmid, and phagemid. Moreover, it can also

amplify by the direct RT-PCR method using what prepared the mRNA fraction from the above-mentioned cell or the above-mentioned organization.

As DNA which carries out the code of the partial peptide of this invention, specifically For example, (1) array number:2, array number:4, array number:6, or an array number : DNA which has the partial base sequence of DNA which has the base sequence expressed with 8, It has the base sequence hybridized under stringent conditions. or (2) array number: — the base sequence expressed with 2, array number:4, array number:6, or array number:8, and a high — array number [ of this invention ]: — HI7T213 containing the amino acid sequence expressed with 1, array number:3, array number:5, or array number:7 — substantial — homogeneous activity (an example —) DNA which has the partial base sequence of DNA which carries out the code of the receptor protein which has ligand avidity, a signal signal transduction operation, etc. is used.

Array number: As DNA which is expressed with 2, array number:4, array number:6, or array number:8 and which can carry out base sequence hybridization, DNA containing the base sequence which has about 95% or more of homology more preferably about 90% or more etc. is preferably used about 80% or more with the base sequence expressed with array number:2, array number:4, array number:6, or array number:8, for example.

The homology of a base sequence is the above mentioned homology computational algorithm NCBI. It is calculable on the same conditions using BLAST.

The approach and conditions of hybridization are the same as that of the above.

[0062]

As a means of cloning of DNA which carries out the code of HI7T213 or the partial peptide (it may be hereafter written as HI7T213 of this invention) of those of this invention completely [ whether it amplifies by the PCR method using the synthetic DNA primer which has the partial base sequence of HI7T213 of this invention, and ]

Or the hybridization of what carried out the indicator of the DNA included in the suitable vector using the DNA fragment or synthetic DNA which carries out the code of the part or all the fields of HI7T213 of this invention can sort out. The approach of hybridization can be performed to Molecular Cloning 2nd (J.Sambrook et al., Cold Spring Harbor Lab.Press, 1989) according to the approach of a publication etc.

Moreover, when using a commercial library, according to the approach of a publication, it can carry out to attached directions for use.

conversion of the base sequence of DNA — PCR, a well-known kit (TAKARA SHUZO CO., LTD.), for example, Mutan<sup>TM</sup>-super Express Km, Mutan<sup>TM</sup>-K (TAKARA SHUZO CO., LTD.), etc. — using — ODA-LA the PCR method and Gapped duplex — law and Kunkel — it can carry out according to the approach according to an approach or them with well-known law etc.

It can be used for a linker, being able to digest DNA which carries out the code of the HI7T213 cloned by remaining as it is for the purpose, and being able to digest it with a restriction enzyme by request, or adding. This DNA may have ATG as a translation initiation codon in the five prime end side, and may have TAA, TGA, or TAG as a translation termination codon in the three-dash terminal side. These translation initiation codons and translation termination codons can also be added using a suitable synthetic DNA adapter.

The expression vector of HI7T213 of this invention can cut down the DNA fragment made into the purpose from DNA which carries out the code of HI7T213 of for example, (b) this invention, and can manufacture it by connecting a (b) this DNA fragment with the lower stream of a river of the promotor in a suitable expression vector.

[0063]

pA 1-11 besides being animal viruses, such as bacteriophages, such as a plasmid (an example, pBR322, pBR325, pUC12, pUC13) of the Escherichia coli origin, a plasmid (an example, pUB110, pTP5, pC194) of the Bacillus-subtilis origin, a yeast origin plasmid (an example, pSH19, pSH15), and lambda phage, a retrovirus, a vaccinia virus, and a baculovirus, etc. as a vector, pXT1, pRc/CMV, pRc/RSV, pcDNAI/Neo, etc. are used.

As long as it is a suitable promotor as a promotor used by this invention corresponding to the host who uses for gene expression, what kind of thing may be used. For example, when using an animal cell as a host, SRalpha promotor, an SV40 promotor, an LTR promotor, a CMV promotor, a HSV-TK promotor, etc. are mentioned.

It is [ among these ] desirable to use a CMV promotor, SRalpha promotor, etc. When a host is the Escherichia bacillus and a trp promotor, a lac promotor, a recA promotor, lambdaPL promotor, a lpp promotor, etc. are [ hosts ] the genus Bacillus, SPO1 promotor, SPO2 promotor, a penP promotor, etc. have PHO5 promotor, a PGK promotor, a GAP promotor, a desirable ADH promotor, etc., when a host is yeast. When a host is an insect cell, a polyhedrin promotor, P10 promotor, etc. are desirable.

[0064]

What contains an enhancer, the splicing signal, the poly A addition signal, the selective marker, the SV40 duplicate origin (it may be hereafter called SV40 ori for short), etc. by the request above else can be used for an expression vector. As a selective marker, a dihydrofolic acid reductase (it may be hereafter called dhfr for short) gene [methotrexate (MTX) resistance], an ampicillin resistance gene (it may be hereafter called Amp<sup>r</sup> for short), a neomycin resistance gene (G418 resistance which may be hereafter called Neor for short), etc. are mentioned, for example. When using a dhfr gene as a selective marker especially using a CHO (dhfr<sup>-</sup>) cell, the purpose gene can be chosen also by the culture medium which does not contain thymidine. Moreover, the signal sequence suitable for a host is added to N terminal side of the receptor protein of this invention if needed. When a host is the Escherichia bacillus, hosts are [ PhoA and a signal sequence, OmpA, a signal sequence, etc. ] the genus Bacillus and an alpha-amylase signal sequence, a subtilisin signal sequence, etc. are [ hosts ] yeast, when a host is an animal cell, an insulin signal sequence, alpha-interferon signal sequence, an antibody molecule, a signal sequence, etc. can use MFalpha and a signal sequence, SUC2, a signal sequence, etc., respectively. Thus, a transformant can be manufactured using the vector containing DNA which carries out the code of HI7T213 of built this invention.

[0065]

As a host, the Escherichia bacillus, the genus Bacillus, yeast, an insect cell, an insect, an animal cell, etc. are used, for example.

As an example of the Escherichia bacillus Escherichia coli () [ Escherichia ] coliK12 and DH1[Proc.Natl. Acad. Sci. USA, 60 volumes, 160 ] and JM103[Nucleic (1968) Journal of Molecular Biology, 120 volumes, and Acids Research, nine-volume, 309(1981)], and JA221 [517 (1978)] HB101[Journal of Molecular Biology, 41 volumes, 459(1969)], C600 [Genetics, 39 volumes, and 440 (1954)], etc. are used.

As the genus Bacillus, it is Gene, 24-volume, and Bacillus subtilis (Bacillus subtilis) MI114 [255 (1983)] 207-21[Journal of, for example. Biochemistry, 95 volumes, 87(1984)], etc. are used.

As yeast, Saccharomyces cerevisiae (Saccharomyces cerevisiae) AH22, AH22R-, and NA87-11A, DKD-5D, 20B-12, Schizosaccharomyces POMBE (Schizosaccharomyces pombe) NCYC1913 and NCYC2036, Pichia pastoris (Pichia pastoris), etc. are used, for example.

[0066]

As an insect cell, when a virus is AcNPV, it is High Five<sup>TM</sup> of the larva origin established cell line (Spodoptera frugiperda cell;Sf cell) of a cabbage armyworm, MG1 cell of the mid gut origin of Trichoplusia ni, and the egg origin of Trichoplusia ni, for example. A cell, the cell of the Mamestra brassicae origin, or the cell of the Estigmena acrea origin is used. When a virus is BmNPV, a silkworm origin established cell line (Bombyx mori N;BmN cell) etc. is used. As this Sf cell, Sf9 cell (ATCC CRL1711), Sf21 cell (above, Vaughn, J.L. et al., in vivo (In Vivo), 13, 213 -217, (1977)), etc. are used, for example.

As an insect, the larva of a silkworm etc. is used, for example [Maeda et al., Nature, 315 volumes, and 592 (1985)].

As an animal cell, ape cell COS-7, Vero, a chinese hamster cell CHO (a following and CHO cell and brief sketch), dhfr genetic defect chinese hamster cell CHO (a following and CHO (dhfr-) cell and brief sketch), a mouse L cell, mouse AtT-20, a mouse myeloma cell, a rat GH 3, a Homo sapiens FL cell, etc. are used, for example.

[0067]

In order to carry out the transformation of the Escherichia bacillus, according to the approach of a publication, it can carry out to Proc.Natl.Acad.Sci.USA, 69 volumes, 2110 (1972) and Gene, 17 volumes, 107 (1982), etc.

In order to carry out the transformation of the genus Bacillus, according to the approach of a publication, it can carry out to Molecular & General Genetics, 168 volumes, 111 (1979), etc.

In order to carry out the transformation of the yeast, according to the approach of a publication, it can carry out to Methods in Enzymology, 194 volumes, 182-187 (1991), Proc.Natl.Acad.Sci.USA, 75 volumes, 1929 (1978), etc.

In order to carry out the transformation of an insect cell or the insect, according to the approach of a publication, it can carry out to Bio/Technology, 6, 47-55 (1988), etc.

for carrying out the transformation of the animal cell -- for example, the cell technology separate volume 8 -- new -- according to the approach of a publication, it can carry out to cell technology experiment protocol .263-267 (1995) (Shujunsha issue), Virology, 52 volumes, and 456 (1973).

Thus, the transformant by which the transformation was carried out by the expression vector containing DNA which carries out the code of HI7T213 is obtained.



In case a host cultivates the transformant which are the *Escherichia bacillus* and the genus *Bacillus*, as a culture medium used for culture, a liquid medium is suitable, and a carbon source required for growth of this transformant, a nitrogen source, and an inorganic substance and others are made to contain in it. As a carbon source, a calcium chloride, a sodium dihydrogenphosphate, a magnesium chloride, etc. are mentioned as nitrogen sources, such as a glucose, a dextrin, soluble starch, and cane sugar, for example, for example as inorganic [ , such as ammonium salt, nitrates, corn steep liquor a peptone, casein, a meat extract, soybean cake, and potato extract ] or an organic substance, and an inorganic substance. Moreover, a yeast extract, vitamins, a growth promoter, etc. may be added. As for pH of a culture medium, about 5-8 is desirable.

[0068]

As a culture medium at the time of cultivating the *Escherichia bacillus*, M9 culture medium [a mirror (Miller), Journal of Experiments in Molecular Genetics, 431-433, Cold Spring Harbor Laboratory, and New York 1972] which contains a glucose and casamino acids, for example is desirable. In order to work a promotor efficiently as occasion demands here, drugs like a 3beta-indolyl acrylic acid can be added.

When a host is the *Escherichia bacillus*, culture can usually be performed at about 15-43 degrees C for about 3 to 24 hours, and aeration and churning can also be added as occasion demands.

When a host is the genus *Bacillus*, culture can usually be performed at about 30-40 degrees C for about 6 to 24 hours, and aeration and churning can also be added as occasion demands.

In case a host cultivates the transformant which is yeast, as a culture medium Burkholder (Burkholder) minimal-medium [Bostian, K.L. et al. [ for example, ], SD culture-medium [Bitter containing Proc.Natl.Acad.Sci.USA, 77 volumes, 4505(1980)], or 0.5% casamino acids, and G.A. \*\* — Proc.Natl.Acad.Sci.USA, 81 volumes, and 5330(1984)] are mentioned. As for pH of a culture medium, adjusting to about 5-8 is desirable. Culture is usually performed at about 20 degrees C - 35 degrees C for about 24 to 72 hours, and aeration and churning are added if needed.

[0069]

In case a host cultivates the transformant which is an insect cell or an insect, what added additives, such as bovine serum, suitably as a culture medium 10% inactivated to Grace's Insect Medium (Grace, T.C.C., Nature, 195, 788 (1962)) is used. As for pH of a culture medium, adjusting to about 6.2-6.4 is desirable. Culture is usually performed for about three - five days at about 27 degrees C, and aeration and churning are added if needed.

In case a host cultivates the transformant which is an animal cell, as a culture medium For example About 5 - 20% of new-born calf serum Included Virology, eight-volume, and Science, 122-volume, and MEM culture-medium [501 (1952)] DMEM culture-medium [396 (1959)] RPMI 1640 culture-medium [The Journal of the American Medical Association, 199 volumes, 519(1967)], 199 culture media [Proceeding of the Society for the Biological Medicine, 73 volumes, and 1 (1950)], etc. are used. As for pH, it is desirable that it is about 6-8. Culture is usually performed at about 30 degrees C - 40 degrees C for about 15 to 60 hours, and

aeration and churning are added if needed.

HI7T213 of this invention can be made to generate out of intracellular [ of a transformant ], a cell membrane, or a cell as mentioned above.

[0070]

In order to carry out separation purification of HI7T213 of this invention from the above-mentioned culture, it can carry out by the following approach.

After facing extracting HI7T213 of this invention from a culture fungus body or a cell, collecting a fungus body or cells by the well-known approach after culture, suspending this in the suitable buffer solution and destroying a fungus body or a cell by the supersonic wave, the lysozyme, freeze thawing, etc., the method of obtaining the crude extract of HI7T213 by centrifugal separation or filtration etc. is used suitably. Protein modifiers, such as a urea and guanidine hydrochloride, and surfactants, such as triton X-100TM, may be contained in the buffer solution. When HI7T213 are secreted in culture medium, a fungus body or a cell, and supernatant liquid are separated by the well-known approach after culture termination, and supernatant liquid is collected.

Thus, purification of HI7T213 contained in the obtained culture supernatant or an extract can be performed, combining appropriately well-known separation and purification method. How to use solubility, such as a salting-out and a solvent precipitation method, as these well-known separation and a purification method, Methods of mainly using the difference of molecular weight, such as dialysis, ultrafiltration, gel filtration, and an SDS-polyacrylamide-gel-electrophoresis method, How to use specific Shinwa nature using the difference of electric charges, such as an ion exchange chromatography, such as an approach and affinity chromatography, The method of using the difference of the isoelectric points, such as an approach of using hydrophobic differences, such as reversed phase high pressure liquid chromatography, and isoelectric focusing, etc. is used.

[0071]

It is convertible for educt or other salts by the approach of applying to a well-known approach or well-known it correspondingly, when it can change into a salt and is conversely obtained with a salt by the approach of applying to a well-known approach or well-known it correspondingly when HI7T213 obtained in this way are obtained by educt.

In addition, by making protein repair enzyme suitable before purification or after purification HI7T213 which recombinant produces act, qualification can be added to arbitration or a polypeptide can also be removed partially. As protein repair enzyme, a trypsin, a chymotrypsin, arginyl proteinase, protein kinase, glycosidase, etc. are used, for example.

The activity of HI7T213 of this invention generated in this way can be measured by the enzyme immunoassay using a joint experiment and a specific antibody with the ligand which carried out the indicator etc.

[0072]

As long as the antibody to HI7T213 of this invention is an antibody which can recognize HI7T213 of this invention, it may be any of a polyclonal antibody and a monoclonal antibody.

The antibody to HI7T213 of this invention can be manufactured according to the

manufacturing method of a well-known antibody or antiserum, using HI7T213 of this invention as an antigen.

[0073]

[Production of a monoclonal antibody]

(a) Production of a monoclonal antibody production cell

The part in which an antibody production is possible is medicated with HI7T213 of this invention with itself or support, and a diluent by administration to mammalian. In order to raise antibody production ability on the occasion of administration, a complete Freund's adjuvant and an incomplete Freund's adjuvant may be prescribed for the patient. Administration is usually performed about a total of 2 to 10 times by a unit of 1 time every 2-6 weeks. As mammalian used, although an ape, a rabbit, a dog, a guinea pig, a mouse, a rat, a sheep, and a goat are mentioned, a mouse and a rat are used preferably, for example.

A monoclonal antibody production hybridoma can be prepared by choosing the individual in which antibody titer was accepted from the homeotherm by which immunity was carried out in the antigen, for example, a mouse, on the occasion of production of a monoclonal antibody production cell, extracting a spleen or lymph gland two - five days after the last immunity, and uniting with a myeloma cell the antibody forming cell contained in them. Measurement of the antibody titer in antiserum can be performed by measuring the activity of the indicator agent combined with the antibody, after making after-mentioned labeling receptor protein and antiserum react. Fusion actuation can be carried out according to a known approach [Nature (Nature), 256 volumes, and 495 pages (1975)], for example, the approach of Kohler and Milstein. As a fusion accelerator, although a polyethylene glycol (PEG), a Sendai virus, etc. are mentioned, PEG is used preferably, for example.

As a myeloma cell, although NS-1, P3U1, SP2/0, etc. are mentioned, P3U1 is used preferably, for example. The desirable ratio of the number of antibody forming cells (spleen cell) and the number of myeloma cells which are used is 1:1 to about 20:1, and it is added by the concentration whose PEG (preferably PEG1000-PEG6000) is about 10 - 80%, and it can carry out efficiently about 20-40 degrees C of cell fusion by incubating for about 1 - 10 minutes at about 30-37 degrees C preferably.

[0074]

Although various approaches can be used for screening of a monoclonal antibody production hybridoma for example, the solid phase (an example —) to which the antigen of receptor protein was made to stick with direct or support The anti-immunoglobulin antibody which added the hybridoma culture supernatant to the microplate and then carried out the indicator with the radioactive substance, the enzyme, etc. (when the cell used for cell fusion is a mouse) Protein A is added or an anti-mouse immunoglobulin antibody is used. A hybridoma culture supernatant is added to the solid phase to which the approach, the anti-immunoglobulin antibody, or protein A which detects the monoclonal antibody combined with solid phase was made to stick. The receptor protein which carried out the indicator with the radioactive substance, an enzyme, etc. is added, and the method of detecting the monoclonal antibody combined with solid phase etc. is mentioned.

Sorting of a monoclonal antibody can be performed by the culture medium for

well-known or the animal cells which usually added HAT (hypoxanthine, aminopterin, thymidine) although it could carry out according to the approach according to it etc. As sorting and a culture medium for breedings, as long as it can grow a hybridoma, what kind of culture medium may be used. For example, RPMI 1640 culture medium which contains 10 – 20% of fetal calf serum preferably, the GIT culture medium (Wako Pure Chem Industry) containing 1 – 10% of fetal calf serum, or the serum free medium for hybridoma culture (SFM-101 and NISSUI PHARMACEUTICAL CO., LTD.) can be used 1 to 20%. 20–40 degrees C of culture temperature are usually about 37 degrees C preferably. Culture time amount is usually one week – two weeks preferably for five days to three weeks. Culture can usually be performed under 5% carbon dioxide gas. The antibody titer of a hybridoma culture supernatant can be measured like measurement of the antibody titer in the above-mentioned antiserum. [0075]

(b) Purification of a monoclonal antibody

Separation purification of a monoclonal antibody can be performed according to the separation purification method [the specific purification method which extracts only an antibody with activity adsorbents, such as an example, a salting-out method, a alcohol precipitation method, isoelectric point settling, an electrophoresis method, the adsorption-and-desorption method by the ion exchanger (an example, DEAE), an ultracentrifugal method, gel filtration, antigen joint solid phase, protein A, or Protein G, is made to dissociate association, and obtains an antibody] of an immunoglobulin like separation purification of the usual polyclonal antibody. [0076]

[Production of a polyclonal antibody]

The polyclonal antibody of this invention can be manufactured according to well-known or the approach according to it. For example, the complex of the immunogen (HI7T213 antigen) and carrier protein is built, immunity is performed to mammalian like the manufacturing method of the above-mentioned monoclonal antibody, the antibody inclusion to HI7T213 of this invention is extracted from this immune animal, and it can manufacture by performing separation purification of an antibody.

It is related with the complex of the immunogen and carrier protein which are used in order to carry out immunity of the mammalian. The class of carrier protein, and the mixing ratio of a carrier and hapten If an antibody is efficiently made to the hapten which the carrier was made to construct a bridge and carried out immunity Although what kind of thing may be made to construct a bridge by what kind of ratio, about 0.1–20 and the method of making it KAPURU [ about one to 5 rate ] preferably are used, for example to hapten 1 by the weight ratio in bovine serum albumin, the cow thyroglobulin, keyhole limpet hemocyanin, etc.

Moreover, although various condensing agents can be used for coupling of hapten and a carrier, the activity ester reagent containing glutaraldehyde, a carbodiimide, maleimide activity ester, a thiol group, and a JICHIOBIRIJIRU radical etc. is used. The part in which an antibody production is possible is medicated with a condensation product with itself or support, and a diluent to a homeotherm. In order to raise antibody production ability on the occasion of administration, a complete Freund's adjuvant and an incomplete Freund's adjuvant may be prescribed for the

patient. administration — usually — every 1 time per about 2–6 weeks, and a total — it can carry out about about 3 to 10 times.

The blood of the mammalian by which immunity was carried out by the above-mentioned approach, ascites, etc. can extract a polyclonal antibody from blood preferably.

Measurement of the polyclonal antibody titer in antiserum can be measured like measurement of the antibody titer in the above-mentioned blood serum. Separation purification of a polyclonal antibody can be performed according to the separation purification method of the same immunoglobulin as separation purification of the above-mentioned monoclonal antibody.

[0077]

The antisense DNA (it may be hereafter written as the antisense DNA of this invention) to DNA of DNA (it may be hereafter written as DNA of this invention) which carries out the code of HI7T213 of this invention and HI7T213, the antibody (it may be hereafter written as the antibody of this invention) to HI7T213, and this invention has the following applications.

[0078]

(1) Physic containing DNA which carries out the code of HI7T213 or HI7T213 DNA which carries out the code of HI7T213 or HI7T213 can be used as physic, such as prevention of a wound, injury of spinal cord, the analgesia, etc. and/or a therapy agent, or a kidney regenerant.

When using DNA which carries out the code of HI7T213 or HI7T213 as above-mentioned physic, it can carry out according to a stock-in-trade. For example, it can be parenterally used in the form of injections, such as water, an axenic solution with the other liquid which can be permitted pharmacologically, or a suspension agent, in taking orally as the tablet and capsule which gave glycolalx and an enteric coat if needed, elixirs, a microcapsule agent, etc. For example, it can manufacture by mixing with this compound or its salt with the unit dosage gestalt required of the medicine manufacture implementation generally accepted with the support and the flavor agent which can be accepted physiologically, an excipient, a vehicle, antiseptics, the stabilizer, the binder, etc. Capacity with the directed range suitable for the amount of active principles in these pharmaceutical preparation is obtained.

Making this patient prescribe for the patient and discover DNA which carries out the code of (b) HI7T213, by transplanting this cell to this patient, after inserting in a (b) cell etc. DNA which carries out the code of HI7T213 and making it discovered, etc., DNA which carries out the code of HI7T213 can make the amount of HI7T213 in this patient's cell able to increase, and can fully demonstrate an operation of ligand. When using DNA which carries out the code of HI7T213, after inserting this DNA in suitable independent or vectors, such as a retrovirus vector, an adenovirus vector, and an adenovirus-associated virus vector, it can carry out according to a stock-in-trade.

[0079]

As an additive which can mix with a tablet, a capsule, etc., a flavor agent like plumping agents, such as gelatin, corn starch, tragacanth gum, a binder like gum arabic, an excipient like a crystalline cellulose, corn starch, gelatin, and an alginic acid, lubricant like magnesium stearate, cane sugar, a lactose or a sweetening agent

like saccharin, peppermint, a dirt mono-oil, or a cherry etc. is used, for example. When dispensing unit form voice is a capsule, liquefied support still like fats and oils can be contained into said type of ingredient. The sterile constituent for injection can prescribe natural appearance vegetable oil, such as an active substance in a vehicle like water for injection, sesame oil, and coconut oil, etc. according to the usual pharmaceutical preparation implementation of making it dissolve or suspend etc. As aqueous liquid for injection, the isotonic solutions (for example, D-sorbitol, D-mannitol, a sodium chloride, etc.) containing the adjuvant of a physiological saline, grape sugar, or others etc. are raised, for example, and you may use together with a suitable solubilizing agent (for example, ethanol), for example, alcohol, polyalcohol (for example, propylene glycol, a polyethylene glycol), a nonionic surfactant (for example, (polysorbate 80 TM) HCO-50), etc. Sesame oil, soybean oil, etc. are raised as oily liquid, and you may use together with benzyl benzoate, benzyl alcohol, etc. as a solubilizing agent.

Moreover, you may blend with a buffer (for example, a phosphate buffer, the sodium acetate buffer solution), aponia-ized agents (for example, a benzalkonium chloride, procaine hydrochloride, etc.), stabilizers (for example, a human serum albumin, a polyethylene glycol, etc.), preservatives (for example, benzyl alcohol, a phenol, etc.), an antioxidant, etc. Suitable ampul is usually filled up with the prepared parenteral solution. Thus, the pharmaceutical preparation obtained is safe, and since it is low toxicity, a medicine can be prescribed for the patient, for example to Homo sapiens or nonhuman homeotherms (for example, a mouse, a rat, a guinea pig, a rabbit, a fowl, a sheep, Buta, a cow, a cat, a dog, an ape, a mantle baboon, a chimpanzee, etc.). For example, although it is different with a symptom etc., in internal use, generally in adult's wound patient, about 0.1-100mg per day of about 1.0-50mg of doses of DNA which carries out the code of HI7T213 or HI7T213 is about 1.0-20mg more preferably (as weight of 60kg). Although a dose changes with the object organ for administration, a symptom, medication methods, etc. once [ the ] when prescribing a medicine for the patient parenterally, it is convenient to prescribe more preferably about about 0.01-30mg [ per day ] about about 0.1-20mg about about 0.1-10mg for the patient by the intravenous injection, for example in administration to adult's wound patient (as the weight of 60kg) in the form of injections. The amount which converted into per weight of 60kg also in other animals can be prescribed for the patient.

[0080]

## (2) Physic containing the antibody to HI7T213

As mentioned above, since the symptoms of cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, and a hyperalgesia etc. may be shown, for the animal which has high-discovered HI7T213, the antibody which has the operation which neutralizes the activity of HI7T213 can be used as prevention of cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, a hyperalgesia, etc., and/or a therapy agent.

The prevention and/or the therapy agent containing the antibody (an example, neutralizing antibody) of this invention of the above-mentioned disease can be prescribed for the patient taking-orally-wise or parenterally as a physic constituent of a suitable pharmaceutical form as liquids and solutions to Homo sapiens or

nonhuman mammals (an example, a rat, a rabbit, a sheep, Buta, a cow, a cat, a dog, ape, etc.) as it is. Although a dose changes with the object disease for administration, a symptom, administration routes, etc. for example, in using it for an adult for the purpose of [ of a cataract ] a therapy The antibody of this invention is made once into an amount. Usually 0.01 – 20 mg/kg weight extent, It is preferably convenient 0.1 – 10 mg/kg weight extent and to prescribe 0.1 – 5 mg/kg weight extent for the patient by the intravenous injection about 1 to 3 times per day preferably about 1 to 5 times per day still more preferably. The amount to which it applies to this also in other parenteral administration and internal use can be prescribed for the patient. When a symptom is especially heavy, you may increase according to the symptom. [0081]

The antibody of this invention can be prescribed for the patient as a suitable itself or physic constituent. The physic constituent used for the above-mentioned administration contains the above or its salt, the support which may be permitted in pharmacology and a diluent, or an excipient. This constituent is offered as dosage forms suitable for taking orally or parenteral administration.

That is, for example, as a constituent for internal use, a tablet (a sugar-coated tablet and a film coated tablet are included), a pill, a granule, powder, a capsule (a soft capsule is included), syrups, an emulsion, suspension, etc. are given to the dosage forms of a solid-state or a liquid, and a concrete target. This constituent is manufactured by the well-known approach, and contains the support, diluent, or excipient usually used in the pharmaceutical preparation field. For example, a lactose, starch, sucrose, magnesium stearate, etc. are used as the support for tablets, and an excipient.

As a constituent for parenteral administration, injections, suppositories, etc. are used and injections include dosage forms, such as an intravenous injection agent, a subcutaneous injection agent, an intradermal injection agent, an intramuscular injection agent, and an intravenous drip infusion agent, for example. These injections are prepared by dissolving, suspending or emulsifying the above-mentioned antibody or its salt in the sterile aqueous or the oily liquid usually used for injections according to a well-known approach. As aqueous liquid for injection, the isotonic solution containing the adjuvant of a physiological saline, grape sugar, or others etc. is used, for example, and you may use together with a suitable solubilizing agent (an example, ethanol), for example, alcohol, polyalcohol (an example, propylene glycol, polyethylene glycol), a nonionic surface active agent [an example, polysorbate 80, and HCO-50 (polyoxyethylene(50 mols) adduct of hydrogenated castor oil)], etc. As oily liquid, sesame oil, soybean oil, etc. are used and benzyl benzoate, benzyl alcohol, etc. may be used together as a solubilizing agent, for example. Suitable ampul is usually filled up with the prepared parenteral solution. The suppositories used for rectum administration are prepared by mixing the above-mentioned antibody or its salt to the usual basis for suppositories.

As for the above-mentioned object for taking orally or the above-mentioned physic constituent for parenteral, it is convenient to be prepared by the dosage forms of a medication unit which suits the dose of an active ingredient. As dosage forms of this medication unit, a tablet, a pill, a capsule, injections (ampul), suppositories, etc. are illustrated, and it is 5–500mg usually per each medication unit dosage forms desirable

in injections that the 10–250mg above-mentioned antibody contains in the dosage forms of 5–100mg and others especially.

In addition, the above mentioned class product may contain other active ingredients, unless the interaction which is not desirable is produced by combination to the above-mentioned antibody.

[0082]

(3) The diagnostic agent containing the antibody to HI7T213

Since the antibody to HI7T213 can recognize HI7T213 specifically, it can be used for the quantum of HI7T213 in sample liquid, especially the quantum by sandwiches immunoassay, etc.

Namely, this invention,

(i) -- the assay of HI7T213 in the sample liquid characterized by measuring the rate of labeled HI7T213 which the antibody of this invention, and sample liquid and HI7T213 labeled were made to react competitively, and were combined with this antibody -- and

(ii) The assay of HI7T213 in the sample liquid characterized by measuring the activity of the indicator agent on insolubilization support for the antibody of this invention which insolubilized on sample liquid and support, and another antibody of labeled this invention coincidence or after making it react continuously is offered.

[0083]

In the assay of the above (ii), one antibody is an antibody which recognizes N edge of HI7T213, and it is desirable that it is the antibody to which the antibody of another side reacts to C edge of HI7T213.

Moreover, the quantum of HI7T213 can be performed using the monoclonal antibody to HI7T213, and also detection by organization dyeing etc. can also be performed.

The antibody molecule itself may be used for these purposes, and F(ab')<sub>2</sub>, Fab', or the Fab fraction of an antibody molecule may be used for them.

Especially the assay of HI7T213 using the antibody of this invention should not be restricted, and as long as it is chemical or a measuring method computed from the standard curve which detected by the physical means and produced this using the standard solution containing the antigen of a known amount about the amount of the antibody corresponding to the amount of antigens in measured liquid (for example, HI7T213 amount), an antigen, or the antibody-antigenic complex, which measuring method may be used for it. For example, although a nephrometry, the competing method, an immunometric method, and a sandwich technique are used suitably, it is desirable especially to use the sandwich technique mentioned later in respect of sensibility and singularity.

[0084]

As an indicator agent used for the measuring method using a marker, radioisotope, an enzyme, a fluorescent material, photogene, etc. are used, for example. As radioisotope, [125I], [131I], [3H], [14C], etc. are used, for example. As the above-mentioned enzyme, it is stable, and the big thing of specific activity is desirable, for example, the beta-galactosidase, the beta-glucosidase, the alkaline phosphatase, a par oxidase, a malate dehydrogenase, etc. are used. As a fluorescent material, fluorescamine, full ORESSEN isothiocyanate, etc. are used, for example. As photogene, luminol, a luminol derivative, luciferin, lucigenin, etc. are used, for example.



Furthermore, a biotin-avidin system can also be used for association with an antibody or an antigen, and an indicator agent.

The approach using the chemical bond used for using physical adsorption, and usually insolubilizing and fixing HI7T213 or an enzyme in insolubilization of an antigen or an antibody may be used. As support, synthetic resin, such as insoluble polysaccharide, such as agarose, a dextran, and a cellulose, polystyrene, polyacrylamide, and silicon, or glass is raised.

The quantum of the HI7T213 amount of this invention in sample liquid can be carried out by making sample liquid react to the monoclonal antibody of this invention which insolubilized in the sandwich technique (primary response), making the monoclonal antibody of another this invention which labeled further react (secondary response), and measuring the activity of the indicator agent after \*\* and on insolubilization support. A primary response and a secondary response may be performed to a reverse order, or may be performed to coincidence, may shift time amount and may perform it. A labeling agent and the approach of insolubilization can apply to above them correspondingly. Moreover, in the immunoassay by the sandwich technique, the number of the antibodies used for the antibody for solid phase or the antibody for indicators does not necessarily need to be one, and they may use the mixture of two or more kinds of antibodies for the purpose of raising sensitometry.

[0085]

In the measuring method of HI7T213 by the sandwich technique of this invention, the antibody in which the part where HI7T213 combine the monoclonal antibody of this invention used for a primary response and a secondary response is different from each other is used preferably. That is, when, as for the antibody used for a primary response and a secondary response, the antibody used by the secondary response recognizes C edge of HI7T213, the antibody which the antibody used by the primary response is desirable, and recognizes N edge except C edge is used.

The monoclonal antibody of this invention can be used for gaging systems, for example, the competing method, immunometric methods, or nephrometries other than a sandwich technique etc.

By the competing method, after making the antigen and labelled antigen in sample liquid react competitively to an antibody, an unreacted labelled antigen (F) and the labelled antigen (B) combined with the antibody are separated (B/F separation), either amount of indicators of B and F is measured, and the quantum of the amount of antigens in sample liquid is carried out. The solid phase-ized method using [ the 1st antibody ] a solid phase-ized antibody as the 2nd antibody using the thing of fusibility is used for this reacting method in B/F separation, using a solid phase-ized antibody as the liquid phase process using the 2nd antibody to a polyethylene glycol and said antibody etc., and the 1st antibody, using a fusibility antibody as an antibody. In an immunometric method, the back solid phase and the liquid phase to which the competitive reaction of the antigen and solid phase-ized antigen in sample liquid was carried out to the labeling antibody of a constant rate are separated, or the antigen in sample liquid and the labeling antibody of an excessive amount are made to react, and after adding a solid phase-ized antigen next and combining an unreacted labeling antibody with solid phase, solid phase and the liquid phase are separated. Next, the amount of indicators of one of phases is measured, and the quantum of the amount

of antigens in sample liquid is carried out.

Moreover, in a nephrometry, the amount of the insoluble sediment produced within gel and in the solution as a result of the antigen-antibody reaction is measured. The amounts of antigens in sample liquid are few, and also when only a small amount of sediment is obtained, the laser nephrometry using dispersion of laser etc. is used suitably.

In applying the immunoassay of these each to the quantum approach of this invention, a setup of special conditions, actuation, etc. is not needed. What is necessary is to add the usual technical consideration of this contractor to the usual conditions in each approach, and operation information, and just to build the system of measurement of HI7T213 of this invention. A total theory, a compendium, etc. can be referred to about the detail of these general technical means.

For example, inlet The volume on \*\* "radioimmunoassay" (Kodansha, Showa 49 issue), Inlet The volume on \*\* "\*\* radioimmunoassay" (Kodansha, Showa 54 issue), the volumes "enzyme immunoassay" (the 2nd edition) (Igaku-Shoin —) for "enzyme immunoassay" (Igaku-Shoin, Showa 53 issue) Eiji Ishikawa edited by Eiji Ishikawa The volumes "enzyme immunoassay" (the 3rd edition) (Igaku-Shoin, Showa 62 issue) Showa 57 issue and for Eiji Ishikawa, "Methods in ENZYMOLOGY"

Vol.70 (Immunochemical Techniques (Part A)), The said document Vol.73 (Immunochemical Techniques (Part B)), The said document Vol.74 (Immunochemical Techniques (Part C)), The said document Vol.84 (Immunochemical Techniques (Part D : Selected Immunoassays)), The said document Vol.92 (Immunochemical Techniques (Part E : Monoclonal Antibodies and General Immunoassay Methods)), said document Vol.121 (above) (Immunochemical Techniques (Part I : Hybridoma Technology and Monoclonal Antibodies)) The Academic Press issue etc. can be referred to.

Sensibility can improve HI7T213 of this invention a quantum by using the antibody of this invention as mentioned above.

[0086]

Furthermore, when reduction of the concentration of HI7T213 is detected by carrying out the quantum of the concentration of HI7T213 using the antibody of this invention, for example, or it is the disease relevant to the malfunction of HI7T213, for example, a wound, injury of spinal cord, and the analgesia, it can be diagnosed that possibility of falling ill in the future is high.

Moreover, when the increment in the concentration of HI7T213 is detected, or it is the disease resulting from the superfluous manifestation of HI7T213, for example, cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, and a hyperalgesia, it can be diagnosed that possibility of falling ill in the future is high.

[0087]

#### (4) Gene-diagnosis agent

DNA which carries out the code of HI7T213 for example, by using it as a probe Homo sapiens or a nonhuman mammal (for example, a rat, a mouse, and a guinea pig —)

Since the abnormalities (abnormality of the genes) of DNA which carries out the code of HI7T213 in a rabbit, a sheep, Buta, a cow, a horse, a cat, a dog, an ape, etc., or mRNA are detectable For example, it is useful as gene-diagnosis agents, such as damage on this DNA or mRNA, mutation or a manifestation fall, and an increment in

this DNA or mRNA or excess of a manifestation.

The above-mentioned gene diagnosis using the antisense polynucleotide to DNA or it which carries out the code of HI7T213 can be carried out by well-known Northern hybridization, the PCR-SSCP method (Genomics, the 5th volume, 874-879 pages (1989), Proceedings of the National Academy of Sciences of the United States of America, the 86th volume, 2766-2770 pages (1989)), etc., for example.

For example, when manifestation reduction of HI7T213 is detected by Northern hybridization, possibility of being diseases, such as a wound, injury of spinal cord, and analgesia, is high, or it can be diagnosed that possibility of falling ill in the future is high.

For example, when the excess of a manifestation of HI7T213 is detected by Northern hybridization, possibility of being diseases, such as cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, and a hyperalgesia, is high, or it can be diagnosed that possibility of falling ill in the future is high.

[0088]

(5) DNA containing an antisense DNA

Since the manifestation of HI7T213 can be controlled, the antisense DNA to DNA which carries out the code of HI7T213 can be used as prevention of cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, a hyperalgesia, etc., and/or a therapy agent.

When using the above-mentioned antisense DNA as the above-mentioned prevention and/or a therapy agent, this antisense DNA can be pharmaceutical-preparation-ized like DNA which carries out the code of HI7T213.

Thus, the pharmaceutical preparation obtained is low toxicity and can be prescribed for the patient taking-orally-wise or parenterally to Homo sapiens or nonhuman mammals (an example, a rat, a rabbit, a sheep, Buta, a cow, a cat, a dog, ape, etc.).

In addition, this antisense DNA can also be prescribed for the patient with a catheter like a gene gun or a hydro gel catheter with support accepted physiologically, such as an adjuvant for promotion of intake, as it is.

Although the dose of this antisense DNA changes with administration roots an object disease and for administration etc., when carrying out partial administration of the antisense DNA for the purpose of the therapy of a cataract at organs (an example, liver, lungs, heart, kidney, etc.), it is about 0.1 to 100 mg per day to an adult (weight of 60kg), for example.

[0089]

Furthermore, like the above-mentioned antisense DNA, the ribozyme containing a part of double stranded RNA (RNAi; RNA interference law) containing a part of RNA which carries out the code of HI7T213, and RNA complementary to it, and RNA which carries out the code of HI7T213 etc. can control the manifestation of DNA which carries out the code of HI7T213, and can control the function of DNA which carries out the code of ligand in the living body or HI7T213.

Therefore, since the manifestation of HI7T213 can be controlled, this double stranded RNA or a ribozyme can be used as prevention of cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, a hyperalgesia, etc., and/or a therapy agent.

Double stranded RNA can be designed and manufactured based on the array of DNA of this invention according to a well-known approach (an example, Nature, 411

volumes, 494 pages, and 2001).

A ribozyme can be designed and manufactured based on the array of DNA of HI7T213 according to a well-known approach (an example, TRENDS in Molecular Medicine, seven volumes, 221 pages, and 2001). For example, it can manufacture by connecting a well-known ribozyme with a part of RNA which carries out the code of HI7T213. The part (RNA fragment) only close to the cutting section on RNA of HI7T213 which may be cut by the well-known ribozyme as a part of RNA which carries out the code of HI7T213 is mentioned.

When using above-mentioned double stranded RNA or an above-mentioned ribozyme as the above-mentioned prevention and/or a therapy agent, it can pharmaceutical-preparation-ize like an antisense DNA, and a medicine can be prescribed for the patient.

[0090]

(6) The screening approach

The screening approach of this invention,

(6A-1) The screening approach of the matter to which the affinity of the ligand characterized by using (i) ligand and (ii) HI7T213 (a partial peptide also being included hereafter) and HI7T213 is changed,

(6A-2) It is the screening approach of the matter of adjusting the amount of manifestations of HI7T213 characterized by using DNA which carries out the code of HI7T213.

[0091]

First, the screening approach of the matter to which the affinity of ligand and HI7T213 is changed is explained.

The matter to which the affinity of ligand and HI7T213 is changed can be screened by building the manifestation system of recombinant HI7T213, using HI7T213, and using the receptor joint assay system using this manifestation system.

HI7T213 are such minded. Cell stimulus activity Isolation for example, arachidonic-acid isolation, acetylcholine isolation, and intracellular calcium<sup>2+</sup> — Intracellular cAMP generation, intracellular cGMP generation, inositol phosphatase production, The phosphorylation of cell membrane potential fluctuation and intracellular protein, activation of c-fos, The matter (namely, HI7T213 agonist) which has the activity which promotes the fall of pH etc., or the activity to control, the matter (namely, HI7T213 antagonist) which does not have this cell stimulus activity are contained. It includes in both the case where "the affinity of ligand and HI7T213 is changed" checks association with ligand and HI7T213, and the case of promoting association with ligand and HI7T213.

[0092]

That is, this invention offers the screening approach of the matter to which the affinity of the ligand characterized by performing the comparison with the case where ligand and a trial compound are contacted to the case where ligand is contacted to (i) HI7T213, and (ii) HI7T213, and HI7T213 is changed.

In the screening approach of this invention, the amount of association of the ligand [ as opposed to HI7T213 ] at the time of contacting ligand and a trial compound, cell stimulus activity, etc. are measured and compared with the case where ligand is contacted to (i) HI7T213, and (ii) HI7T213.

[0093]

Specifically, the screening approach of this invention is,

- (i) The screening approach of the matter to which the affinity of the ligand characterized by measuring and measuring the amount of association to HI7T213 of the ligand the case where the ligand which carried out the indicator is contacted to HI7T213, and at the time of contacting the ligand and the trial compound which carried out the indicator to HI7T213 which carried out the indicator, and HI7T213 is changed,
- (ii) When the ligand which carried out the indicator is contacted to the membrane fraction of the cell containing HI7T213, or this cell, It can set, when the ligand and the trial compound which carried out the indicator are contacted to the membrane fraction of the cell containing HI7T213, or this cell. The amount of association to this cell or this membrane fraction of ligand which carried out the indicator is measured. The screening approach of the matter to which the affinity of the ligand characterized by comparing and HI7T213 is changed, When HI7T213 discovered on the cell membrane by cultivating the transformant containing DNA which carries out the code of HI7T213 for the ligand which carried out the indicator are made to contact,
- (iii) It can set, when HI7T213 discovered on the cell membrane by cultivating the transformant containing DNA which carries out the code of HI7T213 for the ligand and the trial compound which carried out the indicator are made to contact. the ligand which carried out the indicator — this — the screening approach of the matter to which the affinity of the ligand characterized by measuring and measuring the amount of association to HI7T213 and HI7T213 is changed,

[0094]

- (iv) When the compound (for example, ligand, agonist) which activates HI7T213 is contacted into the cell containing HI7T213, It can set, when the compound and trial compound which activate HI7T213 are contacted into the cell containing HI7T213. Cell stimulus activity through HI7T213 (for example, arachidonic acid isolation) Acetylcholine isolation, intracellular calcium<sup>2+</sup> isolation, intracellular cAMP generation, intracellular cGMP generation, Inositol phosphatase production, cell membrane potential fluctuation, the phosphorylation of intracellular protein, the screening approach of the matter to which the affinity of the ligand characterized by measuring and comparing the activity which promotes activation of c-fos, the fall of pH, etc., or the activity to control, and HI7T213 is changed — and
- (v) When HI7T213 discovered on the cell membrane by cultivating the transformant containing DNA which carries out the code of HI7T213 for the compound (for example, ligand, agonist) which activates HI7T213 are made to contact, It can set, when HI7T213 discovered on the cell membrane by cultivating the transformant containing DNA which carries out the code of HI7T213 for the compound and trial compound which activate HI7T213 are made to contact. Cell stimulus activity through HI7T213 (for example, arachidonic acid isolation) Acetylcholine isolation, intracellular calcium<sup>2+</sup> isolation, intracellular cAMP generation, intracellular cGMP generation, Inositol phosphatase production, cell membrane potential fluctuation, the phosphorylation of intracellular protein, It is the screening approach of the matter to which the affinity of the ligand characterized by measuring and comparing the activity which promotes activation of c-fos, the fall of pH, etc., or the activity to

control, and HI7T213 is changed etc.

[0095]

Concrete explanation of the screening approach of this invention is given below. First, although you may be which thing as long as it contains the above mentioned HI7T213 as HI7T213 used for the screening approach of this invention, Homo sapiens, the membrane fraction of the organ of a nonhuman homeotherm, etc. are suitable. However, since especially the organ of the Homo sapiens origin is very difficult to receive, HI7T213 which carried out the extensive manifestation, using recombinant as what is used for screening are suitable.

What is necessary is just to follow the below-mentioned method of preparation in the screening approach of this invention, when using a cell or this cell membrane fraction containing HI7T213 etc.

When using the cell containing HI7T213, this cell may be fixed with glutaraldehyde, formalin, etc. The fixed approach can be performed according to a well-known approach.

Although the host cell which discovered HI7T213 is said as a cell containing HI7T213, as this host cell, the above-mentioned Escherichia coli, a Bacillus subtilis, yeast, an insect cell, an animal cell, etc. are mentioned.

As membrane fraction, after crushing a cell, the thing of the fraction in which many cell membranes obtained by the well-known approach are contained is said. Crushing by making a cell blow off from a thin nozzle etc. is mentioned pressurizing as the crushing approach of a cell by crushing by the approach and Waring blender which crush a cell with a Potter-Elvehjem mold homogenizer, or the poly TRON (product made from Kinematica), crushing by the supersonic wave, an French press, etc. The fractionation method by centrifugal forces, such as a differential centrifugation method and density gradient centrifugation, is mainly used for the fractionation of a cell membrane. For example, centrifugal [ of the cell crushing liquid / short-time (usually about 1 minute - 10 minutes) ] is carried out at a low speed (500rpm - 3000rpm), usually carry out centrifugal [ of the supernatant liquid ] further for 30 minute - 2 hours at high speed (15000rpm - 30000rpm), and let precipitation obtained be membrane fraction. In this membrane fraction, many discovered membrane components, such as phospholipid of HI7T213 and the cell origin and membrane protein, are contained.

As for the amount of HI7T213 in the cell containing this HI 7T213, or membrane fraction, it is desirable that it is 103 to 108 molecule per cell, and it is suitable for it that it is 105 to 107 molecule. In addition, the ligand avidity per membrane fraction (specific activity) becomes high, and construction of a high sensitivity screening system is not only attained, but it can measure a lot of samples with the same lot, so that there are many amounts of manifestations.

[0096]

In order to carry out the aforementioned (i) which screens the matter to which the affinity of ligand and HI7T213 is changed - (iii), the ligand which carried out the indicator to HI7T213 suitable fraction is used. Recombinant HI7T213 fraction which has activity equivalent to HI7T213 fraction of a natural mold or it as HI7T213 fraction is desirable. Here, equivalent ligand avidity etc. is indicated to be equivalent activity. As ligand which carried out the indicator, the ligand which carried out the indicator,

the ligand analog compound which carried out the indicator are used. For example, the ligand by which the indicator was carried out by [ $^3\text{H}$ ], [ $^{125}\text{I}$ ], [ $^{14}\text{C}$ ], [ $^{35}\text{S}$ ], etc. can be used.

In order to specifically screen the matter to which the affinity of ligand and HI7T213 is changed, a receptor preparation is prepared by suspending the membrane fraction of the cell which contains HI7T213 first, or a cell in the buffer suitable for screening. Any are sufficient as long as it is the buffer which does not check association with ligands, such as a phosphoric-acid buffer of pH 4–10 (desirably pH 6–8), and a tris-hydrochloric-acid buffer, and HI7T213 in a buffer. Moreover, surface active agents, such as CHAPS, Tween-80TM (Kao-atlas company), digitonin, and a deoxycholate, can also be added to a buffer in order to reduce nonspecific association. Furthermore, protease inhibitors, such as PMSF, leupeptin, E-64 (made in a peptide lab), and pepstatin, can also be added in order to suppress the decomposition of HI7T213 or ligand by the protease. The ligand in which the constant rate (5000cpm – 500000cpm) carried out the indicator to this HI7T213 0.01ml – 10ml solution is added, and the trial compound of  $10^{-4}$  –  $10^{-1}$ microM is made to live together in coincidence. In order to know the amount (NSB) of nonspecific association, the reaction tube which added the ligand of the non-indicator of an overlarge is also prepared. About 50 degrees C of reactions are desirably performed from about 30 minutes from about 20 minutes at about 4 to about 37 degrees C from about 0 degree C for about 3 hours for about 24 hours. It filters through a glass fiber filter paper etc. after a reaction, and after washing by this buffer of optimum dose, the radioactivity which remains in a glass fiber filter paper is measured at a liquid scintillation counter or gamma-counter. When the count (B0-NSB) which subtracted the amount (NSB) of nonspecific association from the count (B0) in case there is no matter which rivals is made into 100%, the trial compound with which the amount (B-NSB) of specific bindings becomes 50% or less can be chosen as candidate matter with antagonistic inhibition capacity.

[0097]

In order to enforce the aforementioned approach of (iv)– (v) which screens the matter to which the affinity of ligand and HI7T213 is changed Cell stimulus activity through HI7T213 (for example, arachidonic acid isolation) Acetylcholine isolation, intracellular calcium $^{2+}$  isolation, intracellular cAMP generation, intracellular cGMP generation, The activity which promotes the phosphorylation of inositol phoshate production, cell membrane potential fluctuation, and intracellular protein, activation of c-fos, the fall of pH, etc., or the activity to control can be measured using a well-known approach or the commercial kit for measurement. Specifically, the cell containing HI7T213 is first cultivated on a multi-well plate etc. After exchanging for the suitable buffer which does not show toxicity to a fresh culture medium or a fresh cell beforehand in screening, adding a trial compound etc. and carrying out fixed time amount incubation, according to each approach, the quantum of the product which collected and generated the cell for an extract or digestive liquor is carried out. When assay with the dialytic ferment which a cell contains is difficult for the generation of matter (for example, arachidonic acid etc.) made into the index of cell stimulus activity, the inhibitor to this dialytic ferment may be added and assay may be performed. Moreover, about activity, such as cAMP production control, it is

detectable as production depressant action to the cell which increased the amount of fundamental production of a cell by forskolin etc.

In order to screen by measuring cell stimulus activity, the cell which discovered HI7T213 [ suitable ] is required. As a cell which discovered HI7T213 of this invention, the above-mentioned recombinant HI7T 213 manifestation cell strain etc. is desirable.

As a trial compound, a peptide, protein, a nonpeptidic compound, a synthetic compound, a fermentation product, a cell extract, a vegetable extract, an animal tissue extract, plasma, etc. may be mentioned, for example, and these compounds may be new compounds and may be well-known compounds. A trial compound has the desirable acid addition salt which the salt may be formed, and a salt with acids (the example, inorganic acid, etc.), bases, etc. (an example, organic acid, etc.) which are permitted physiologically is used as a salt of a trial compound, and is especially permitted physiologically. As such a salt, a salt with inorganic acids (for example, a hydrochloric acid, a phosphoric acid, a hydrobromic acid, a sulfuric acid, etc.) or a salt with organic acids (for example, an acetic acid, a formic acid, a propionic acid, a fumaric acid, a maleic acid, a succinic acid, a tartaric acid, a citric acid, a malic acid, oxalic acid, a benzoic acid, methansulfonic acid, benzenesulfonic acid, etc.) is used, for example.

[0098]

the kit for screening of the matter to which the affinity of ligand and HI7T213 is changed contains the membrane fraction of the cell containing HI7T213 and HI7T213, or this cell, and (or) ligand.

The following are mentioned as an example of the kit for screening of this invention.

#### 1. Reagent for Screening

(i) The buffer solution for measurement, and the buffer solution for washing  
What added 0.05% of bovine serum albumin (sigma company make) to Hanks' Balanced Salt Solution (Gibco make).

or it carries out filtration sterilization with the filter of 0.45 micrometers of apertures and saves at 4 degrees C — or business — the time — you may prepare .

(ii) HI7T213 preparation

What carried out the passage of the CHO cell which made HI7T213 discover to 12 hole plate in 5x105 pieces / hole, and cultivated it for two days by 37 degrees C, 5%CO<sub>2</sub>, and 95%air.

(iii) Indicator ligand

Ligand which carried out the indicator by [3H], [125I], [14C], [35S], etc.

what was dissolved in a suitable solvent or the suitable buffer solution — 4 degrees C or -20 degrees C — saving — business — it sometimes dilutes with the buffer solution for measurement at 1microM.

(iv) Ligand standard solution

Ligand is dissolved so that it may be set to 1mM by PBS which contains bovine serum albumin (sigma company make) 0.1%, and it saves at -20 degrees C.

[0099]

#### 2. Measuring Method

(i) After 1ml of buffer solutions for measurement washes twice the cell which made HI7T213 cultivated on the plate for 12 hole tissue culture discover, the buffer



solution for measurement of 490microl is added to each hole.

(ii) After 5microl Adding the trial compound solution of 10<sup>-3</sup>-10<sup>-10</sup>M, the ligand which carried out the indicator is 5microl Added, and it is made to react at a room temperature for 1 hour. 5microl In order to know the amount of nonspecific association, add the ligand of 10<sup>-3</sup>M instead of the trial compound.

(iii) Reaction mixture is removed and the 1ml buffer solution for washing washes 3 times. It is 0.2Ns about the indicator ligand combined with the cell. It dissolves by NaOH-1%SDS and mixes with 4ml liquid-scintillator A (Wako Pure Chem make).

(iv) Radioactivity is measured using a liquid scintillation counter (made in Beckmann), and Percent Maximum Binding (PMB) is calculated by the following formula [several 1].

[A-one number]

$$\text{PMB} = [(B - \text{NSB}) / (B_0 - \text{NSB})] \times 100$$

PMB:Percent Maximum Binding

B : the value when adding a specimen

NSB:Non-specific Binding (the amount of nonspecific association)

B<sub>0</sub> : The amount of the maximum association

[0100]

The matter obtained using the screening approach of this invention or the kit for screening is matter to which association with ligand and HI7T213 is changed (association is checked or promoted), and is matter (HI7T213 so-called antagonist) which does not have the matter (HI7T213 so-called agonist) or this stimulus activity which specifically has cell stimulus activity through HI7T213.

the peptide chosen from said trial compound carried out, protein, a nonpeptidic compound, a synthetic compound, a fermentation product, etc. may be mentioned, these compounds may be new compounds and this matter may be a well-known compound. The concrete evaluation approach of whether it is HI7T213 agonist or to be an antagonist should just follow the following (i) - (iv).

(i) For example, binding assay shown by the screening approach of the above (i) - (iii) is performed, and after obtaining the matter to which the affinity of ligand and HI7T213 is changed (association is checked especially), it measures whether it has the cell stimulus activity through HI7T213 which this matter described above. The matter which has cell stimulus activity is HI7T213 agonist, and the matter which does not have this activity is HI7T213 antagonist.

(ii) (a) trial compound is contacted into the cell containing HI7T213, and the cell stimulus activity through the above-mentioned HI7T213 is measured. The matter which has cell stimulus activity is HI7T213 agonist.

(b) Measure and compare the cell stimulus activity through HI7T213 the case where the compounds (for example, ligand etc.) which activate HI7T213 are contacted into the cell containing HI7T213, and at the time of contacting the compound and trial compound which activate HI7T213 into the cell containing HI7T213. The matter which may decrease cell stimulus activity with the compound which activates HI7T213 is HI7T213 antagonist.

(iii) A trial compound is applied to the nonhuman mammal of (a) this invention, or its part, and HI7T 213 agonist activity is authorized. The matter which has agonist activity is HI7T213 agonist.

(b) Apply a trial compound to the nonhuman mammal of this invention, or its part, and authorize the improvement effect or the kidney playback effectiveness of a disease, such as a wound, injury of spinal cord, or analgesia. The matter with which the improvement effect or the kidney playback effectiveness of this disease was accepted is HI7T213 agonist.

(iv) A trial compound is applied to the nonhuman mammal of this invention which has shown the symptoms of diseases, such as the above-mentioned phenotype or cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, and a hyperalgesia, or its part, and the improvement effect of the above-mentioned phenotype or a disease is authorized. The matter with which the improvement effect of the above-mentioned phenotype or a disease was accepted is HI7T213 antagonist.

[0101]

since this HI7T213 agonist can activate HI7T213 like the ligand to HI7T213 — safe — low — it is useful as physic, such as prevention of a toxic wound and injury of spinal cord, the analgesia, etc. and/or a therapy agent, or a kidney regenerant. on the contrary — since HI7T213 antagonist can control the activation of HI7T213 by ligand — safe — low — toxic cancer and renal dysfunction, a cataract, dermatitis, a chronic pain, and nociception — it is useful as physic, such as which sensitive prevention and/or a therapy agent.

The matter (an example, agonist, antagonist) obtained using the above-mentioned screening approach or the kit for screening may form the salt, for example, a permissible salt etc. is used pharmacologically. Specifically, a salt with an inorganic base, a salt with an organic base, a salt with an inorganic acid, a salt with an organic acid, basicity, or acidic amino acid etc. is raised.

As a suitable example of a salt with an inorganic base, alkaline-earth-metal salts, such as alkali-metal salts, such as sodium salt and potassium salt, a calcium salt, and magnesium salt, and an aluminum salt, ammonium salt, etc. are raised, for example.

As a suitable example of a salt with an organic base, it is \*\*\*\*\* with trimethylamine, triethylamine, pyridine, picoline, 2, 6-lutidine, ethanolamine, diethanolamine, triethanolamine, cyclohexylamine, dicyclohexylamine, N, and N'-dibenzyl ethylenediamine etc., for example.

As a suitable example of a salt with an inorganic acid, a salt with a hydrochloric acid, a hydrobromic acid, a sulfuric acid, a phosphoric acid, etc. is raised, for example.

As a suitable example of a salt with an organic acid, a salt with a formic acid, an acetic acid, a propionic acid, a fumaric acid, oxalic acid, a tartaric acid, a maleic acid, a citric acid, a succinic acid, a malic acid, methansulfonic acid, benzenesulfonic acid, a benzoic acid, etc. is raised, for example.

As a suitable example of a salt with a basic amino acid, a salt with an arginine, a lysine, ORUCHININ, etc. is raised, for example, and a salt with an aspartic acid, glutamic acid, etc. is raised as a suitable example with acidic amino acid, for example.

[0102]

When using the matter obtained using the screening approach of this invention, or the kit for screening as above-mentioned physic, it can pharmaceutical-preparation-ize like the physic constituent containing above HI7T213.

For example, although it is different with a symptom etc., in internal use, generally in

adult's wound patient, about 0.1–100mg per day of about 1.0–50mg of doses of HI7T213 agonist is about 1.0–20mg more preferably (as weight of 60kg). Although a dose changes with the object organ for administration, a symptom, medication methods, etc. once [ the ] when prescribing a medicine for the patient parenterally, it is convenient to prescribe more preferably about about 0.01–30mg [ per day ] about about 0.1–20mg about about 0.1–10mg for the patient by the intravenous injection, for example in administration to adult's wound patient (as the weight of 60kg) in the form of injections. The amount which converted into per weight of 60kg also in other animals can be prescribed for the patient.

On the other hand, although it is different with a symptom etc., in internal use, generally in adult's cataract patient, about 0.1–100mg per day of about 1.0–50mg of doses of HI7T213 antagonist is about 1.0–20mg more preferably (as weight of 60kg). Although a dose changes with the object organ for administration, a symptom, medication methods, etc. once [ the ] when prescribing a medicine for the patient parenterally, it is convenient to prescribe more preferably about about 0.01–30mg [ per day ] about about 0.1–20mg about about 0.1–10mg for the patient by the intravenous injection, for example in administration to adult's cataract patient (as the weight of 60kg) in the form of injections. The amount which converted into per weight of 60kg also in other animals can be prescribed for the patient.

[0103]

Next, the screening approach of the matter of adjusting the amount of manifestations of HI7T213 is explained.

The screening approach of this invention is the screening approach of the matter which specifically promotes or checks the manifestation of HI7T213 characterized by to measure and measure the amount of mRNA(s) which carries out the code of each amount of manifestations or ligand of HI7T213 at the time of cultivating the cell or organization which may discover (i) HI7T213 under existence of a trial compound and nonexistence.

As the cell which may discover HI7T213, or an organization Homo sapiens and a nonhuman homeotherm (for example, a guinea pig, a rat, a mouse, and a fowl —) cells (for example, a nerve cell —), such as a rabbit, Buta, a sheep, a cow, and an ape An endocrine cell, a neuroendocrine cell, a neuroglia, a pancreas beta cell, a bone marrow cell, Hepatocyte, splenic cells, a mesangial cell, an epidermal cell, an epithelial cell, an endothelial cell, fibrocyte, a desmacyte, a muscle cell, a fat cell, and immunocyte (an example and a macrophage —) A T cell, a B cell, a spontaneous killer cell, a mast cell, neutrophil leucocyte, basophilic leucocyte, Eosinophile leucocyte, monocyte, a dendritic cell, megakaryocyte, a synovial cell, chondrocyte, osteocyte, Osteoblast, an osteoclast, an alveolar epithelial cell, an interstitial cell, or the precursor cell of these cells, All the organizations where those cells, such as a stem cell or a gun cell, exist, about (an example, an olfactory bulb, an amygdaloid nucleus, and a cerebrum — the base — a ball, a hippocampus, and a thalamus —) each part of a brain and a brain Hypothalamus, the cerebral cortex, a medulla oblongata, a cerebellum, a spine, a hypophysis, the stomach, the pancreas, the kidney, Liver, a gonad, the thyroid, the gallbladder, bone marrow, a suprarenal gland, the skin, muscles, lungs, an alimentary canal (an example, the large intestine, small intestine), a blood vessel, the heart, a thymus gland, a spleen, salivary glands,

peripheral blood, a prostate gland, a testis (testis), the ovary, a placenta, a uterus, a bone, a cartilage, a joint, skeletal muscle, etc. may be used. An established cell line and a primary culture system may be used in that case. Moreover, the transformation object by which the transformation was carried out by the recombination vector containing DNA which carries out the code of the above mentioned HI7T213 may be used.

The culture approach of the cell which may discover HI7T213 is the same as that of the cultivation of the above mentioned transformation object.

As a trial compound, a DNA library besides the aforementioned trial compound etc. can be used.

[0104]

The amount of manifestations of HI7T213 is a NOZAN hybridization method, RT-PCR, and TaqMan about mRNA which can also measure by well-known approaches, such as immunochemical method, using an antibody etc., and carries out the code of the ligand. It can also measure by the well-known approach using the PCR method.

the approach of applying to a well-known approach or well-known it correspondingly, in order to measure the amount of manifestations of mRNA by the hybridization method -- for example, molecular -- according to the approach of a publication etc., it can carry out to - cloning (Molecular Cloning) 2nd (J.Sambrook et al., Cold Spring Harbor Lab.Press, 1989).

Measurement of the amount of mRNA which specifically carries out the code of HI7T213 contacts RNA extracted from the cell according to the well-known approach, and DNA which carries out the code of HI7T213, its part or the antisense polynucleotide of this invention, and is performed by measuring the amount of mRNA combined with DNA which carries out the code of HI7T213, its part, or the antisense polynucleotide of this invention. The amount of mRNA which combined HI7T213 with DNA which carries out a code, its part, or the antisense polynucleotide of this invention can measure easily by carrying out the indicator of DNA which carries out the code of HI7T213, its part, or the antisense polynucleotide of this invention with radioisotope, coloring matter, etc. As radioisotope,  $^{32}\text{P}$ ,  $^3\text{H}$ , etc. are used, for example, and fluorochromes, such as fluorescein, FAM (product made from PE Biosystems) and JOE (product made from PE Biosystems), TAMRA (product made from PE Biosystems), and ROX (product made from PE Biosystems), Cy5 (product made from Amersham), Cy3 (product made from Amersham), are used as coloring matter, for example.

Moreover, the amount of mRNA can be performed by measuring the amount of cDNA amplified by PCR using DNA which carries out the code of HI7T213, its part, or the antisense polynucleotide of this invention as a primer, after changing into cDNA RNA extracted from the cell with reverse transcriptase.

Thus, it can choose as matter which has the activity which checks the manifestation of HI7T213 for the trial compound which decreases the amount of mRNA which can choose as matter which has the activity which promotes the manifestation of HI7T213 for the trial compound to which the amount of mRNA which carries out the code of HI7T213 is made to increase, and carries out the code of HI7T213.

[0105]

Furthermore, this invention,

(ii) The screening approach of the matter which promotes or checks the promotor activity concerned characterized by measuring and comparing each reporter activity at the time of cultivating the transformant which carried out the transformation under existence of a trial compound and nonexistence with the lower stream of a river of the promoterregion of the gene which carries out the code of HI7T213, or an enhancer field by the recombinant DNA which connected the reporter gene is offered.

As a reporter gene, lacZ (beta-galactosidase gene), chloramphenicol acetyltransferase (CAT), luciferase, a growth factor, the beta-glucuronidase, the alkaline phosphatase, Green fluorescent protein (GFP), a beta lactamase, etc. are used, for example.

By measuring the amount of a reporter gene product (an example, mRNA, protein) using a well-known approach, it can choose as the matter which has the operation which controls the promotor of HI7T213 of this invention, or activity of an enhancer for the trial compound to which the amount of a reporter gene product is made to increase (it promotes especially), i.e., matter which has the activity which promotes the manifestation of HI7T213. On the contrary, it can choose as the matter which has the operation which controls the promotor of HI7T213, or activity of an enhancer for the trial compound which decreases the amount of a reporter gene product (it prevents especially), i.e., matter which has the activity which checks the manifestation of HI7T213.

The same thing as the above is used as a trial compound.

Culture of a transformant can be performed like the aforementioned transformant. Vector construction and the assay method of a reporter gene can follow a well-known technique (for example, Molecular Biotechnology 13, 29-43, 1999).

[0106]

The matter which has the activity (activity to which the amount of manifestations is made to increase) which promotes the manifestation of HI7T213 can be used as physic, such as prevention of a wound, injury of spinal cord, the analgesia, etc. and/or a therapy agent, or a kidney regenerant.

The matter which has the activity (activity which decreases the amount of manifestations) which checks the manifestation of HI7T213 can be used as physic, such as prevention of cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, a hyperalgesia, etc., and/or a therapy agent.

The matter obtained using the screening approach of this invention is matter chosen from the above mentioned trial compound. The same thing as the salt of the matter obtained as a salt of the matter obtained using the screening approach of this invention using the screening approach of of the above mentioned agonist or the above mentioned antagonist is used.

When using the matter obtained using the screening approach of this invention as above-mentioned prevention and/or a therapy agent, it can carry out according to a stock-in-trade. For example, it can consider as a tablet, a capsule, elixirs, a microcapsule agent, an axenic solution, a suspension agent, etc. like the physic constituent containing the above mentioned ligand.

Thus, the pharmaceutical preparation obtained is safe, and since it is low toxicity, a

medicine can be prescribed for the patient to Homo sapiens or nonhuman homeotherms (for example, a mouse, a rat, a rabbit, a sheep, Buta, a cow, a horse, Tori, a cat, a dog, an ape, a chimpanzee, etc.), for example.

Although it is different with the administration route the operation, an object disease, and for administration etc., generally the dose of this matter prescribes more preferably about 0.1–100mg of about 1.0–50mg of about 1.0–20mg of these matter for the patient per day in an adult (per weight of 60kg), when, administering orally the matter which promotes the amount of manifestations of HI7T213 for the purpose of [ of a wound ] a therapy for example. Although the 1-time dose of this matter changes with object diseases for administration etc. when prescribing a medicine for the patient parenterally For example, when an adult (per weight of 60kg) is usually medicated with the matter which promotes the amount of manifestations of HI7T213 for the purpose of [ of a wound ] a therapy in the form of injections, It is convenient per day to prescribe more preferably about about 0.01–30mg about about 0.1–20mg about about 0.1–10mg for the patient for this matter by the intravenous injection. The amount which converted into per weight of 60kg also in other animals can be prescribed for the patient.

On the other hand, when administering orally the matter which checks the amount of manifestations of HI7T213 for the purpose of [ of a cataract ] a therapy, generally in an adult (per weight of 60kg), about 0.1–100mg of about 1.0–50mg of about 1.0–20mg of these matter is more preferably prescribed for the patient per day. Although the 1-time dose of this matter changes with object diseases for administration etc. when prescribing a medicine for the patient parenterally For example, when an adult (per 60kg) is usually medicated with the matter which checks the amount of manifestations of HI7T213 for the purpose of [ of a cataract ] a therapy in the form of injections, It is convenient per day to prescribe more preferably about about 0.01–30mg about about 0.1–20mg about about 0.1–10mg for the patient for this matter by the intravenous injection. The amount which converted into per weight of 60kg also in other animals can be prescribed for the patient.

Such physic can be used pharmaceutical-preparation-izing it like the physic containing the above mentioned HI7T213.

[0107]

#### (7) Knock out animal

This invention offers the nonhuman mammal embryonic stem cell by which DNA of this invention was inactivated, and the DNA manifestation insufficient nonhuman mammal of this invention.

Namely, this invention,

(i) Nonhuman mammal embryonic stem cell by which DNA of this invention was inactivated,

(ii) Embryonic stem cell given in (\*\* i) term inactivated when this DNA introduced a reporter gene (an example, beta-galactosidase gene of the Escherichia coli origin),

(iii) The embryonic stem cell given in (\*\* i) term which is neomycin resistance,

(iv) Embryonic stem cell given in (\*\* i) term a given nonhuman mammal is a GETSU gear-tooth animal,

(v) Embryonic stem cell given in a \*\* (iv) term a given GETSU gear-tooth animal is a mouse,

- (vi) This DNA manifestation insufficient nonhuman mammal by which DNA of this invention was inactivated,
- (vii) The nonhuman mammal given in a \*\* (vi) term which it is inactivated when this DNA introduces a reporter gene (an example, beta-galactosidase gene of the Escherichia coli origin), and this reporter gene may discover under a promotor's control to DNA of this invention,
- (viii) the nonhuman mammal given in a \*\* (vi) term a given nonhuman mammal is a GETSU gear-tooth animal, and the nonhuman mammal given in a \*\* (viii) term given
- (ix) GETSU gear-tooth animal is a mouse — and
- (x) An animal given in a \*\* (vii) term is medicated with a trial compound, and the screening approach of the compound which promotes or checks the promotor activity over DNA of this invention characterized by detecting the manifestation of a reporter gene, or its salt is offered.

[0108]

With the nonhuman mammal embryonic stem cell by which DNA of this invention was inactivated By adding variation to DNA of this invention which this nonhuman mammal has artificially By making the activity of HI7T213 of this invention in which controls the manifestation ability of DNA or this DNA is carrying out the code lose substantially DNA says the embryonic stem cell (it is hereafter written as an embryonic stem cell) of the nonhuman mammal (the knock out DNA of this invention may be called hereafter) which does not have the manifestation ability of HI7T213 of this invention substantially.

The same thing as the above is used as a nonhuman mammal.

As an approach of adding variation to DNA of this invention artificially, it can carry out by making this a part of DNA array or all deletion, and other DNA insert or permute by the gene engineering-technique, for example. What is necessary is just to produce the knock out DNA of this invention by shifting the reading frame of a codon or destroying the function of a promotor or an exon by these variation.

[0109]

As an example of a nonhuman mammal embryonic stem cell (it is hereafter written as the DNA inactivation embryonic stem cell of this invention, or the knock out embryonic stem cell of this invention) in which DNA of this invention was inactivated For example, DNA of this invention which the target nonhuman mammal has is isolated. The drug resistance gene which makes representation a neomycin resistance gene and a hygromycin tolerance gene at the exon part, Or [ whether the function of an exon is destroyed by inserting the reporter gene which makes a lacZ gene and a cat gene representation, and ] Or by inserting the DNA arrays (for example, poly A addition signal etc.) which make the intron part between exons end the imprint of a gene, and carrying out by the ability not compounding perfect mRNA The DNA strand which has the DNA array built so that a gene might be destroyed as a result It introduces into the chromosome of this animal for example, by the homologous [ (which is hereafter written as a targetting vector) ] rearranging method. About the obtained embryonic stem cell, on DNA of this invention Or it analyzes by the PCR method which made the primer the DNA array on the Southern hybridization analysis which used the DNA array of the near as the probe, or a targetting vector, and the DNA array of near fields other than DNA of this invention

used for targetting vector production. It can obtain by sorting out the knock out embryonic stem cell of this invention.

Moreover, what could use the already established above things, for example as an embryonic stem cell of the origin to which inactivation of the DNA of this invention is carried out by the homologous rearranging method etc., and was newly established according to the approach of well-known Evans and Kaufman may be used. For example, in the case of the embryonic stem cell of a mouse, the embryonic stem cell of 129 systems is used for current and a general target, but For the purpose of a genetic background acquiring a clear embryonic stem cell immunologically by the pure line strain replaced with this, since the immunological background has not clarified, for example the little of C57BL/6 mouse or the number of egg gathering of C57BL/6 — the intersection of DBA/2 — what was established using BDF1 mouse (F1 of C57BL/6, and DBA/2) improved more coarsely can be used good. the advantage that BDF1 mouse has many egg gathering, and its egg is strong — in addition, since it has against the background of C57BL/6 mouse, the embryonic stem cell obtained using this can be advantageously used at the point which can replace the genetic background with C57BL/6 mouse by carrying out a back cross with C57BL/6 mouse, when a symptoms model mouse is created.

Moreover, although the blastocyst on the 3.5th is generally used after fertilization when establishing an embryonic stem cell, the eggs of 8 cell term germ can be gathered in addition to this, and many early embryos can be efficiently acquired by cultivating and using to a blastocyst.

moreover, a sex — although which embryonic stem cell may be used, it is convenient for the direction of a male embryonic stem cell usually creating a germ cell line chimera. Moreover, also in order to reduce the time and effort of complicated culture, it is desirable to distinguish a sex as early as possible.

[0110]

As the judgment approach of the sex of an embryonic stem cell, the approach of amplifying the gene of the sex determination field on a Y chromosome, and detecting by the PCR method, can raise as one of them, for example. If this approach is used, since it will end with the number of embryonic stem cells of 1 colony extent (about 50 pieces) to having taken the number of cells of about  $10^6$  pieces to carry out karyotype analysis conventionally, it is possible to perform the first selection of the embryonic stem cell in the early stages of culture by distinction of a sex, and the time and effort in early stages of culture can be sharply reduced by having enabled selection of a male cell at an early stage.

Moreover, as the second selection, the check of the chromosome number by the G-banding method etc. can perform, for example. Although 100% of the normal number of the chromosome number of the embryonic stem cell obtained is desirable, when a relation top, such as physical actuation in the case of establishment, is difficult, after knocking out the gene of an embryonic stem cell, it is desirable to carry out cloning to a normal cell (for example, cell whose chromosome number is  $2n=40$  with a mouse) again.

Thus, although it is usually very good, since the obtained embryonic stem cell strain tends to lose the capacity which can carry out ontogeny, it needs to carry out subculture carefully. [ of the fecundity ] For example, it is the inside of a



carbon-dioxide-gas incubator (preferably) under LIF (1-10000U/ml) existence on a suitable feeder cell like STO fibrocyte. It cultivates by the approach of cultivating at about 37 degrees C 5% with carbon dioxide gas, 95% air or 5% oxygen, 5% carbon dioxide gas, and 90% air. At the time of a passage For example, it single-cell-izes by the trypsin / EDTA solution (usually 0.001-0.5% trypsin / 0.1-5mM EDTA, preferably about 0.1% trypsin / 1mM EDTA) processing, and the approach of carrying out seeding on the newly prepared feeder cell etc. is taken. Although such a passage is usually performed day by day [ 1-3 ], when a cell is observed on this occasion and a cell unusual in gestalt is able to see, to abandon that cultured cell is desired.

By carrying out suspended cell culture until it carries out monolayer culture of it according to suitable conditions until an embryonic stem cell results in high density, or it forms a cell cluster It is possible to make it specialize in the cell of various types, such as a top-of-the-head muscle, a visceral muscle, and a myocardium. [M.J.Evans and M.H.Kaufman, the 292nd volume (Nature) of Nature, 154 pages, 1981;G.R.Martin, Proc.Natl.Acad.Sci.U.S.A., the 78th volume, 7634 pages, and 1981;T.C.Doetschman \*\* -- Journal of embryology and experimental morphology, The 87th volume, 27 pages, 1985], and the DNA manifestation insufficient cell of this invention which the embryonic stem cell of this invention is made to specialize, and is obtained are useful in cell biological examination of this invention in in vitro one of HI7T213.

[0111]

The DNA manifestation insufficient nonhuman mammal of this invention can be distinguished from the normal animal by measuring the amount of mRNA(s) of this animal using the well-known approach, and measuring the amount of manifestations indirectly.

The same thing as the above is used as this nonhuman mammal.

The DNA manifestation insufficient nonhuman mammal of this invention can make DNA of this invention knock out, when the targetting vector produced as mentioned above, for example is introduced into a mouse embryonic stem cell or a mouse ootid and the DNA array in which DNA of this invention of a targetting vector was inactivated by installation carries out homologous recombination which replaces DNA of this invention on the chromosome of a mouse embryonic stem cell or a mouse ootid by gene homologous recombination.

The cell by which DNA of this invention was knocked out can be judged in the analysis by the PCR method which made the primer the Southern hybridization analysis which used the DNA array of the DNA top of this invention, or its near as the probe or the DNA array on a targetting vector, and the DNA array of near fields other than DNA of this invention of the mouse origin used for the targetting vector. When a nonhuman mammal embryonic stem cell is used, the chimera germ which carried out cloning of the cell strain by which DNA of this invention was inactivated, injected the cell into the nonhuman mammal germ or blastocyst of a suitable stage, for example, 8 cells, term, and was produced by gene homologous recombination is transplanted to the uterus of this nonhuman mammal that carried out pseudopregnancy. The created animal is a chimera animal which consists of both a cell with the DNA seat of normal this invention, and a cell with the DNA seat of this invention which varied artificially.

When it has the DNA seat of this invention to which a part of reproductive cell of this chimera animal varied, it is obtained from the population obtained by crossing such a chimera individual and a normal individual by sorting out the individual which consisted of cells with the DNA seat of this invention to which all organizations added variation artificially by the judgment of for example, a coat color etc. Thus, the obtained individual is a hetero manifestation insufficient individual of the peptide of this invention, can cross the hetero manifestation insufficient individual comrade of the peptide of this invention, and can usually obtain the gay manifestation insufficient individual of the peptide of this invention from those offspring.

[0112]

When using an ootid, by pouring in a DNA solution by the microinjection method into an ootid nucleus, the transgenic nonhuman mammal which introduced the targetting vector in the chromosome can be obtained, and it is obtained by choosing what has variation in the DNA seat of this invention by gene homologous recombination compared with these transgenic nonhuman mammals.

Thus, the animal individual obtained by mating can also check that this DNA is knocked out, and the individual by which DNA of this invention is knocked out can perform a breeding passage for it in the usual breeding environment.

Furthermore, what is necessary is just to follow a conventional method also about acquisition and maintenance of a germ cell line. That is, the homozygote animal which has this inactivation DNA in both homologues can be acquired by crossing the animal of the sex which this inactivation DNA holds. The obtained homozygote animal can be efficiently obtained by breeding in the condition that it becomes the normal individual 1 and homozygote plurality, to a mother animal. By crossing the sex of a heterozygote animal, the propagation passage of the homozygote which has this inactivation DNA, and the heterozygote animal is carried out.

The nonhuman mammal embryonic stem cell by which DNA of this invention was inactivated is very useful when creating the DNA manifestation insufficient nonhuman mammal of this invention.

Moreover, since the DNA manifestation insufficient nonhuman mammal of this invention carries out deletion of the various bioactive which may be guided with the peptide of this invention and it can serve as a model of the illness which considers inactivation of the bioactive of the peptide of this invention as a cause, it is useful to cause investigation of these illnesses and examination of a cure.

[0113]

(7a) The screening approach of the matter of having prevention and/or a curative effect to the illness resulting from a deficit, damage, etc. on DNA of this invention The DNA manifestation insufficient nonhuman mammal of this invention can be used for screening of the matter which has prevention and/or a curative effect to the illness resulting from a deficit, damage, etc. on DNA of this invention.

That is, this invention offers the screening approach of the matter of having prevention and/or a curative effect, or the kidney playback effectiveness to the illness resulting from a deficit, damage, etc. on DNA of this invention which medicates the DNA manifestation insufficient nonhuman mammal of this invention with a trial compound, and is characterized by observing and measuring change of this animal, for example, a wound, injury of spinal cord, the analgesia, etc.

The same thing as the above is raised as a DNA manifestation insufficient nonhuman mammal of this invention used in this screening approach.

The same thing as the above is used as a trial compound.

The DNA manifestation insufficient nonhuman mammal of this invention can be processed with a trial compound, and, specifically, prevention and/or the curative effect of a trial compound can be examined as compared with a non-processed control animal by making change of the symptom of each organ of this animal, an organization, and the illness etc. into an index.

As an approach of processing a trial animal with a trial compound, internal use, an intravenous injection, etc. are used and it can choose suitably in accordance with the symptom of a trial animal, the property of a trial compound, etc., for example.

Moreover, the dose of a trial compound can be suitably chosen in accordance with the property of a medication method and a trial compound etc.

[0114]

In this screening approach, when a trial animal is medicated with a trial compound, symptoms, such as a wound of this trial animal, injury of spinal cord, and analgesia, can choose this trial compound about 30% or more preferably about 10% or more as matter which has prevention and/or a curative effect to the above-mentioned disease, when improved about 50% or more more preferably.

Specifically, this trial compound can be used as physic, such as prevention of a wound, injury of spinal cord, the analgesia, etc. and/or a therapy agent, or a kidney regenerating.

The matter obtained using this screening approach is matter chosen from the above-mentioned trial compound, and since it has prevention and/or a curative effect to the disease caused by a deficit, damage, etc. on HI7T213, it can be used as physic, such as \*\*\*\* which it is safe and is the low toxicity, a therapy agent, etc. to this disease. Furthermore, the compound guided from the matter obtained by the above-mentioned screening can be used similarly.

[0115]

The matter obtained by this screening approach has the desirable acid addition salt which the salt may be formed, and a salt with acids (the example, the inorganic acid, organic acid, etc.), bases, etc. (an example, alkali metal, etc.) which are permitted physiologically is used as a salt of this matter, and is especially permitted physiologically. As such a salt, a salt with inorganic acids (for example, a hydrochloric acid, a phosphoric acid, a hydrobromic acid, a sulfuric acid, etc.) or a salt with organic acids (for example, an acetic acid, a formic acid, a propionic acid, a fumaric acid, a maleic acid, a succinic acid, a tartaric acid, a citric acid, a malic acid, oxalic acid, a benzoic acid, methansulfonic acid, benzenesulfonic acid, etc.) is used, for example. The physic containing the matter obtained by this screening approach can be manufactured like the physic containing the above mentioned HI7T213.

Thus, the pharmaceutical preparation obtained is safe, and since it is low toxicity, it can be prescribed for the patient to Homo sapiens or nonhuman mammals (for example, a rat, a mouse, a guinea pig, a rabbit, a sheep, Buta, a cow, a horse, a cat, a dog, an ape, etc.), for example.

About 0.1–100mg of about 1.0–50mg of about 1.0–20mg of these compounds is more preferably prescribed [ in / generally / although the dose of this matter is different

with the administration route for an object disease and administration etc., when administering this matter orally for the purpose of the therapy of a wound, for example / an adult patient (as the weight of 60kg) ] for the patient per day. Although the 1-time dose of this matter changes with object diseases for administration etc. when prescribing a medicine for the patient parenterally For example, when an adult patient is usually medicated with this matter in the form of injections for the purpose of [ of a wound ] a therapy (as weight of 60kg), It is convenient per day to prescribe more preferably about about 0.01–30mg about about 0.1–20mg about about 0.1–10mg for the patient for this matter by the intravenous injection. The amount which converted into per weight of 60kg also in other animals can be prescribed for the patient.

[0116]

(7b) It is the screening approach about the matter which promotes or checks a promotor's activity over DNA of this invention.

This invention medicates the DNA manifestation insufficient nonhuman mammal of this invention with a trial compound, and offers the screening approach of the matter which promotes or checks a promotor's activity over DNA of this invention characterized by detecting the manifestation of a reporter gene.

In the above-mentioned screening approach, as a DNA manifestation insufficient nonhuman mammal of this invention, it is inactivated when DNA of this invention introduces a reporter gene, and what this reporter gene may discover under a promotor's control to DNA of this invention is used also in the DNA manifestation insufficient nonhuman mammal of above mentioned this invention.

The same thing as the above is raised as a trial compound.

The same thing as the above is used as a reporter gene, and a lacZ gene, a fusibility alkaline-phosphatase gene, or a luciferase gene is suitable.

A promotor's activity is detectable by tracing the manifestation of the matter with which a reporter gene carries out the code of the DNA of this invention since a reporter gene exists under a promotor's rule to DNA of this invention in the DNA manifestation insufficient nonhuman mammal of this invention permuted by the reporter gene.

For example, when a part of DNA field which carries out the code of the peptide of this invention is permuted with the lacZ gene of the Escherichia coli origin, originally the beta-galactosidase is discovered in the organization which the peptide of this invention discovers instead of the peptide of this invention. The manifestation condition of this invention the animal in the living body of HI7T213 is observable simple by dyeing using the reagent which follows, for example, serves as a substrate of beta-galactosidase like 5-BUROMO-4-chloro-3-indolyl-beta-galactopyranoside (X-gal). What is necessary is to be the stain solution which fixes the HI7T 213 deficit mouse or its organization intercept of this invention by glutaraldehyde etc., and specifically contains X-gal after washing with a phosphoric-acid buffer physiological salt solution (PBS), to be a room temperature or near 37 degree C, to stop a beta-galactosidase reaction and just to observe coloration by washing the preparation with 1 mM EDTA/PBS solution, about 30 minutes thru/or after making it react for 1 hour. Moreover, according to a conventional method, mRNA which carries out the code of the lacZ may be detected.

The matter obtained using the above-mentioned screening approach is matter chosen from the above-mentioned trial compound, and is matter which promotes or checks the promotor activity over DNA of this invention.

The matter obtained by this screening approach has the desirable acid addition salt which the salt may be formed, and a salt with acids (the example, inorganic acid, etc.), bases, etc. (an example, organic acid, etc.) which are permitted physiologically is used as a salt of this matter, and is especially permitted physiologically. As such a salt, a salt with inorganic acids (for example, a hydrochloric acid, a phosphoric acid, a hydrobromic acid, a sulfuric acid, etc.) or a salt with organic acids (for example, an acetic acid, a formic acid, a propionic acid, a fumaric acid, a maleic acid, a succinic acid, a tartaric acid, a citric acid, a malic acid, oxalic acid, a benzoic acid, methansulfonic acid, benzenesulfonic acid, etc.) is used, for example.

[0117]

Since the manifestation of HI7T213 can be promoted and the function of HI7T213 can be promoted, the matter which promotes the promotor activity over DNA of this invention can be used as physic, such as prevention of a wound, injury of spinal cord, the analgesia, etc. and/or a therapy agent, or a kidney regenerant, for example.

On the other hand, since the manifestation of HI7T213 can be checked and the function of HI7T213 can be checked, the matter which checks the promotor activity over DNA of this invention can be used as physic, such as prevention of cancer, renal dysfunction, a cataract, dermatitis, a chronic pain, a hyperalgesia, etc., and/or a therapy agent, for example.

Furthermore, the matter guided from the matter obtained by the above-mentioned screening can be used similarly.

[0118]

The physic containing the matter obtained by this screening approach can be manufactured like the physic containing the above mentioned ligand.

Thus, the pharmaceutical preparation obtained is safe, and since it is low toxicity, it can be prescribed for the patient to Homo sapiens or nonhuman mammals (for example, a rat, a mouse, a guinea pig, a rabbit, a sheep, Buta, a cow, a horse, a cat, a dog, an ape, etc.), for example.

About 0.1-100mg of about 1.0-50mg of about 1.0-20mg of these matter is more preferably prescribed [ in / generally / when administering orally the matter which promotes promotor / as opposed to / although the dose of this matter is different with the administration root for an object disease and administration etc. / for example / DNA of this invention at the therapy purpose of a wound / activity / an adult patient (as the weight of 60kg) ] for the patient per day. Although the 1-time dose of this matter changes with object diseases for administration etc. when prescribing a medicine for the patient parenterally For example, when an adult patient is usually medicated with the matter which promotes the promotor activity over DNA of this invention for the purpose of [ of a wound ] a therapy in the form of injections (as weight of 60kg), It is convenient per day to prescribe more preferably about about 0.01-30mg about about 0.1-20mg about about 0.1-10mg for the patient for this matter by the intravenous injection. The amount which converted into per weight of 60kg also in other animals can be prescribed for the patient.

On the other hand, when administering orally the matter which checks the promotor

activity over DNA of this invention for the purpose of [ of a cataract ] a therapy, generally in an adult patient, about 0.1–100mg of about 1.0–50mg of about 1.0–20mg of these matter is more preferably prescribed for the patient per day (as weight of 60kg). Although the 1–time dose of this matter changes with object diseases for administration etc. when prescribing a medicine for the patient parenterally For example, when an adult patient is usually medicated with the matter which checks the promotor activity over DNA of this invention for the purpose of [ of a cataract ] a therapy in the form of injections (as weight of 60kg), It is convenient per day to prescribe more preferably about about 0.01–30mg about about 0.1–20mg about about 0.1–10mg for the patient for this matter by the intravenous injection. The amount which converted into per weight of 60kg also in other animals can be prescribed for the patient.

[0119]

Thus, the DNA manifestation insufficient nonhuman mammal of this invention is very useful when screening the matter which promotes or checks a promotor's activity over DNA of this invention, and it can contribute to cause investigation of the various diseases resulting from the DNA manifestation incompetence of this invention or prevention, and/or development of a remedy greatly.

Moreover, if the gene which carries out the code of the various proteins to the lower stream of a river is connected using DNA containing the promoterregion of HI7T213 of this invention, this is poured into the ootid of an animal and the so-called transgenic animal (gene transfer animal) is created, the peptide will be made to compound specifically and it will also become possible to consider an operation with the living body. The still more suitable reporter gene for the above-mentioned promotor part is combined, and if a cell strain which this discovers is established, it can be used as a retrieval system of a low molecular weight compound with the operation which promotes or controls specifically the production capacity in the inside of the body of the peptide of this invention itself.

[0120]

The array number of the array table of this invention shows the following arrays.

[ -- array number: -- 1] -- the amino acid sequence of Homo sapiens HI7T213 is shown.

[ -- array number: -- 2] -- the base sequence of cDNA which carries out the code of Homo sapiens HI7T213 (hHI7T213) is shown.

[ -- array number: -- 3] -- 169th Asp of the amino acid sequence which shows the amino acid sequence of Homo sapiens HI7T213, and is expressed with array number:1 is the amino acid sequence which changed to Asn.

[ -- array number: -- 4] -- the base sequence of cDNA which carries out the code of Homo sapiens HI7T213 (hHI7T213) is shown.

[ -- array number: -- 5] -- the amino acid sequence of mouse HI7T213 (#11) is shown.

[ -- array number: -- 6] -- the base sequence of cDNA which carries out the code of mouse HI7T213 (#11) is shown.

[ -- array number: -- 7] -- the amino acid sequence of mouse HI7T213 (#8) is shown.

[ -- array number: -- 8] -- the base sequence of cDNA which carries out the code

of mouse HI7T213 (#8) is shown.

- [ -- array number: -- 9] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 1 is shown.
- [ -- array number: -- 10] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 1 is shown.
- [ -- array number: -- 11] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 1 is shown.
- [ -- array number: -- 12] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 1 is shown.
- [ -- array number: -- 13] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 2 is shown.
- [ -- array number: -- 14] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 2 is shown.
- [ -- array number: -- 15] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 4 is shown.
- [ -- array number: -- 16] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 4 is shown.
- [ -- array number: -- 17] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 6 is shown.
- [ -- array number: -- 18] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 6 is shown.
- [ -- array number: -- 19] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 6 is shown.
- [ -- array number: -- 20] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 8 is shown.
- [ -- array number: -- 21] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 8 is shown.
- [ -- array number: -- 22] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 8 is shown.
- [ -- array number: -- 23] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 15 is shown.
- [ -- array number: -- 24] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 15 is shown.
- [ -- array number: -- 25] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 15 is shown.
- [ -- array number: -- 26] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 15 is shown.
- [ -- array number: -- 27] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 15 is shown.
- [ -- array number: -- 28] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 15 is shown.
- [ -- array number: -- 29] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 15 is shown.
- [ -- array number: -- 30] -- the base sequence of the primer used for the PCR method performed in the below-mentioned example 15 is shown.
- [ -- array number: -- 31] -- the base sequence of the primer used for the PCR

method performed in the below-mentioned example 15 is shown.

[0121]

In this application specification, when displaying a base, amino acid, etc. by the cable address, based on the cable address by IUPAC-IUB Commission on Biochemical Nomenclature, or the common use cable address in the field concerned, the example is given to a degree.

DNA : deoxyribonucleic acid

cDNA : complementary deoxyribonucleic acid

A : adenine

T : thymine

G : guanine

C : cytosine

RNA : ribonucleic acid

mRNA : messenger RNA

dATP : deoxyadenosine triphosphoric acid

dTTP : deoxythymidine triphosphoric acid

dGTP : deoxyguanosine triphosphoric acid

dCTP : deoxycytidine triphosphoric acid

ATP : adenosine triphosphate

EDTA : ethylenediaminetetraacetic acid

SDS : sodium dodecyl sulfate

Gly : glycine

Ala : alanine

Val : valine

Leu : leucine

Ile : isoleucine

Ser : serine

Thr : threonine

Cys : cysteine

Met : methionine

Glu : glutamic acid

Asp : aspartic acid

Lys : lysine

Arg : arginine

His : histidine

Phe : phenylalanine

Tyr : tyrosin

Trp : tryptophan

Pro : proline

Asn : asparagine

Gln : glutamine

pGlu : pyroglutamic acid

Me : methyl group

Et : ethyl group

Bu : butyl

Ph : phenyl group



TC : thiazolidine-4(R)-carboxamide radical  
[0122]

Moreover, the substituent, protective group, and reagent by which pressure of business is carried out in this specification are written with the following notation.

Tos :p-toluene sulfonyl

CHO : formyl

Bzl : benzyl

Cl2Bzl : 2, 6-dichloro benzyl

Bom : benzyloxymethyl

Z : benzyloxycarbonyl

Cl-Z :2-chloro benzyloxycarbonyl

Br-Z :2-BUROMO benzyloxycarbonyl

Boc : t-butoxycarbonyl

DNP : dinitrophenol

Trt : trityl

Bom :t-butoxy methyl

Fmoc :N-9-fluorenyl methoxycarbonyl

HOBt : 1-hydroxy benzotriazole

HOObt : 3 and 4-dihydro-3-hydroxy-4-oxo--  
1, 2, 3-benzotriazine

HONB :1-hydroxy-5-norbornene -2, 3-JIKARUBOKI  
SHIIMIDO

DCC : N and N'-dicyclohexylcarbodiimide  
[0123]

Transformant Escherichia coli JM109/pCAG 213-1 obtained in the below-mentioned example 2 is 1-1-1, Higashi, Tsukuba-shi, Ibaraki-ken from October 15, 2002. The independent administrative agency National Institute of Advanced Industrial Science and Technology of a center 6th (zip code 305-8566) It is the deposition number FERM to a patent living thing deposition pin center,large. It \*\*\*\*s as BP-8207.  
[0124]

Although an example is given to below and this invention is more concretely explained to it, it cannot be overemphasized that this invention is not limited to these.

[Example 1]

[0125]

The manifestation in the organization of mouse mold hHI7T213 gene

In order to acquire mouse mold hHI7T213 gene, plaque hybridization was performed to the mouse genomic library (129-/SvJ, Toyobo) by using hHI7T213 gene as a probe. The salt concentration of last 0.5xSSC washed after hybridization at 65 degrees C, and it obtained the single plaque (#8 array number : 8 #11 array number:6). The gene expression of the immanency in each organization was investigated among the obtained genes about #8 gene (equivalent to mMrgA6), and #11 gene (similar to mMrgA4) ( drawing 1 ).

RT-PCR Southern hybridization was performed about C57BL / 6 mouse (Charles River Japan) each organization. That is, from each organization of a dorsal spinal nerve root ganglion, a brain, a small intestine, muscles, the heart, a cortex, the

kidney, liver, a spleen, and a testis, all RNA was prepared and reverse transcriptase processing was performed. Next, the reactant which performed PCR with the primer set (CGLP primer: PIWY primer: TGCCTGTCTGT(G/A) CT(G/A) TGCCC(G/T) ATCTGGTAT (array number: 9) and GGGCAA(C/T) CC(G/A) CAGAGGA(G/A) AAAAACCAAAA (array number: 10)) based on an amino acid sequence (CLSV(M/L) CPIWY and LVF(L/F) LGGLP) common to #8 and #11 was moved to the membrane filter after electrophoresis. Southern hybridization was performed to the transferred filter by having used the oligo DNA (#11 probe: #8 probe: TACCAAATATGAAGATGACTATGG (array number: 11), GGTCCCAAATATGTAATTGACTCT (array number: 12)) equivalent to an amino acid sequence (#11: #8: TKYEDDYG, GPKYVIDS) respectively peculiar to both factors as the probe, and the organization which both factors have discovered was questioned. At this time, 2xSSC solutions performed washing after hybridization on the indicator of a probe at 42 degrees C using Oligo dT tailing kit (Roche). # The manifestation was accepted in the brain from the juvenile period, and eight genes were clearly discovered by the maturation period brain and the testis. To it, from the juvenile period, the clear manifestation was accepted by the dorsal spinal nerve root ganglion, and, otherwise, #11 gene was discovered by the maturation period brain, muscles, the heart, and the testis.

From the above results, the fact discovered besides a dorsal spinal nerve root ganglion became clear in the Mrg gene cluster in a mouse.

[Example 2]

[0126]

Construction of the expression vector for transgenic rat creation

The expression vector for transgenic rat creation and pCAG 213-1 were built based on the conventional method ( drawing 2 ).

The DNA fragment (array number: 4) with which this plasmid carries out the code of the hHI7T213 gene is inserted in the EcoRI site of pCXN2. Production of hHI7T213 gene and the contents of the plasmid pCXN2 were shown in following (1) and (2).

(1) hHI7T213 gene : the 970bp fragment which performed the PCR method and cut the obtained fragment by EcoRI using the primer of

TTGAATTGCGCCACCATGGATTCAACCATCCCAGTCT (array number: 13) and TTGAATTCTTATCACTGCTCCAATCTGCTTCCCGACAGCT (array number: 14) to DNA which carries out the code of the Homo sapiens origin hHI7T213 gene (array number : 4 JP,2000-166576,A).

(2) pCXN2 : the plasmid of overall-length 5900bp which has a CAG promotor (1700bp) including a fowl actin promotor, the field (700bp) containing a rabbit globin poly A addition signal, an SV40 duplicate initiation field, an ampicillin resistance gene, and a neomycin resistance gene.

The transformation of the pCAG 213-1 was carried out to Escherichia coli JM 109, and Escherichia coli JM109/pCAG 213-1 was obtained. On the occasion of transgenic rat creation, after cutting pCAG 213-1 by SalI and PvuII, the fragment of 3370bp(s) made into the purpose by the partial digestion of BamHI was obtained.

[Example 3]

[0127]

Production of the transgenic rat which introduced hHI7T213

It is intraperitoneal administration about PMSG of 30IU to the female rat for egg gathering (a Wistar network, 9 weeks old). It carries out and is hCG of after breeding and 5IU for two days in the breeding room of 12-hour \*\* term / 12-hour dark term. It injected intraperitoneally and was made to cross with a male rat (a Wistar network, 12 weeks old). Separately, it is \*\*. It is vasoligature male RATSU about the \*\*\*\*\* rat (a Wistar network, 9 to 13 weeks old) which is carrying out \*\*\*\*\*. It was made to cross with TO (a Wistar network, 10 weeks old or more). On the next day, mating is checked by vaginal plug formation. Open the abdominal cavity of the female rat for egg gathering carried out, and an oviduct is taken out. The HER culture medium which contains FCS 20% (3.180g (Dainippon Pharmaceutical) of HAM-F12 powder culture media) 1.040g (Dainippon Pharmaceutical) of RPMI-1640 powder culture media, and MEM 0.950g (Dainippon Pharmaceutical) of Eagle powder culture media NaHCO<sub>3</sub> (Wako Pure Chem)0.780g, Penicillin-G (GIBCO BRL) 50000U and 50000U500ml of Streptomycin(s) (GIBCO BRL) While dissolving in distilled water, The fertilized egg was taken out with the pincettes under the stereoscopic microscope. A cumulus cell can be taken. HER containing 20%FCS which sucked up \*\*\*\*\* with the pipet and was covered by the mineral oil It is under CO<sub>2</sub> 5% 37 degrees C until it puts in during the drop of a culture medium and carries out injection. It cultivated. It puts in during the drop of PBS containing 20%FCS covered by the mineral oil in the fertilized egg. Suction immobilization was carried out with the holding pipet. Injection \*\*\*\*\* prepared in the example 2 Liquid (10microg/(ml)) is attracted to an injection pipet, and it is INJIE under a stereoscopic microscope. The cushion pipet was thrust into the male pronucleus of a fertilized egg, and the injection fragment was poured in. HE containing 20%FCS covered by the mineral oil in the fertilized egg after injection termination It puts in during the drop of R culture medium, and is under CO<sub>2</sub> 5% 37 degrees C to transplantation to \*\*\*\*\*. It cultivated.

It is after anesthesia and back with Nembutal about the \*\*\*\*\* rat which checked the pseudopregnancy condition by vaginal plug formation. The section was cut open, the pincette drew [ did not gather the fat lump, ] him out, and it fixed by KUREMME. Stereo micro A pincette tears the bursa ovarica under a mirror and it is a transfer pipet about 8-13 fertilized eggs. It used and poured into oviduct opening. 12 after returning the ovary and an oviduct to the inside of the body and suturing a cut end Breeding was continued at the breeding room of \*\*\*\*\* term / 12-hour dark term. 22 days after making an embryo transfer into zero day The \*\* rat (F0) was produced. Transfer of the introductory gene to a \*\* rat is 1cm about a tail. It cuts off with extent scissors, DNA is isolated from this organization extract with a conventional method, and it is the PCR method. It checked.

F0 individual by which transfer of an introductory gene was checked is Wistar in the phase whose reproduction was attained. It was made to cross with a rat and offspring (F1) was obtained. Transfer of an introductory gene is checked like the above. Sib mating of the F1 individuals which have an introductory gene is carried out, and it is related with an introductory gene, and is gay junction. The body was acquired.

[Example 4]

[0128]

The gene analysis of a transgenic rat

it amounted to 4 weeks old — coming out — B.Hogan et al. from the tail of offspring

(Manipulating The Mouse Embryo [ ] --) It is business about 1986 and DNA extracted by the approach of Cold Spring Harvor Laboratories. It is. The primer designed based on the base sequence of hHI7T213 gene (example 2) ( 5'-TGAGCTTCACGGGGCTGACGTGCATCGTTT-3' (array number: 15)), And PCR was performed using 5'-TTGGCAC TGCTGTTAAGAGCGGACAGGAAA-3' (array number: 16). Sum total PC which has detected the PCR fragment of 720bp as a result of analyzing the offspring rat of 168 individuals R positivity individuals were six individuals.

It is by the Southern hybridization method about the genomic DNA of the PCR positivity individual of these 6 individual. It analyzed. That is, DNA of 5microg is completely cut by EcoRI, and it is 1.0%. It moved to the nylon filter after agarose gel electrophoresis. About this filter, it is an example. It is DIG about the DNA fragment containing hHI7T213 gene obtained by 2. RNA labbe ring key It is high BURIDA the probe which carried out the indicator by TTO (the Roche diagnostics company make), and overnight. IZU is carried out, 2xSSC and 0.1%SDS wash twice at a room temperature, and then they are 0.1xSSC(s). SDS washed twice at 68 degrees C 0.1%. The DIG fluorescence detection kit (the Rossi YU diagnostics company make) was used for detection. Consequently, all of these 6 individual are hHI. The fragment of 970bp(s) of the 7T213 origin is checked, and installation of hHI7T213 gene is \*\*. The private seal was carried out. moreover, the copy number of the introduced gene -- 13M line -- 30 copies and 82F a network -- 1 or less \*\*\*\*\* and 90F line -- 1 or less \*\*\*\*\* and 92M line -- one copy -- with -- 96M line is [ the bottom and / five copies and 148M line ] one copies -- a check -- \*\*\*\*.

[Example 5]

[0129]

Transgenic rat acquisition of heterozygote

It is Wistar when the network (the 1st generation (F0)) obtained in the example 4 amounts to 12 weeks old. (Japanese Clare) It crossed with the network rat and acquisition of the second generation (F1) was tried. 4 weeks old When it reaches, PCR is carried out to an example 4 by the approach of a publication, and heterozygote is selected and carried out. It is 13M, 96M, and 148M that the second generation was acquired although used for Example 5. They were only three lines.

[Example 6]

[0130]

TaqMan analysis of hHI7T213mRNA of transgenic rat each network

Brain extracted from the 8-weeks old transgenic rat (F1) obtained in the example 5 The heart, the kidney, and about 500mg are homogenized in ISOGEN (NIPPON GENE make). It carried out and total RNA was extracted with the conventional method. total RNA 5microg is used. First strand cDNA synthesis kit (the Amersham Pharmacia Biotech K.K. make) It used, cDNA composition was performed according to the protocol, and it considered as the template. TaqMan As a primer of analysis, for detection of hHI7T213 gene Forward primer ( 5'-TCCTGTCCGCTCTTAACAGCA-3' (array number: 17)), Reverse primer (5' -TTTTGACGCTGCCTAAAGGAG-3' (array number: 18)), And a FAM indicator TaqMan p -set is used and it is TaqMan analysis (). Etching primer of rimer (5'-TGCCAACCCCATCATTTACTTCTTCGTG-3' (array number: 19)) [ TaqMan7700,

[ Applied ] It is a line about Biochemicals. \*\*\*\*. In detection of G3 PDH which is an internal standard, it is rodent G3 PDH. The set (Applied Biochemicals) was used. Moreover, PCR performed in the example 5 as electronegative control The individual judged to be a wild type was used. hHI7T213 gene expression is 13M. It accepted by all the organs of 96M and 148M system ( drawing 3 ). 96M and 13 Although the pattern of manifestation was the same, it was thought that the amount of manifestations had more 96M as for M. \*\* To \*\*, compared with 13M and 96M, it is few, and the amount of manifestations of 148M is \*\*. Since the present formats also differed, the effect by the insertion location was suggested.

[Example 7]

[0131]

The description observation of a transgenic rat

The symptoms of a cataract and rough hair are shown in MF13M and 961 individual acquired in the example 5. Although the individual to carry out was seen, the frequency and extent were high in 96M. As opposed to it Abnormalities were not accepted in 148M with the low amount of gene expression. Moreover, at 96M, it is after the birth. A skin exanthema transient as with a peak of near 7 ages in day and desquamation were accepted ( drawing 4 ). \*\* The individual which produces waste is a rose in extent of the desquamation in an each object although it was 50% or more of offspring. There was with ( drawing 4 a-b). the above results — hHI7T 213 overgene manifestation RA It is thought that the examples of a type of the phenotype of TTO are 13M and 96M, and a symptom is clear. from — 96M were mainly used for future analyses.

[Example 8]

[0132]

Organization distribution of hHI7T213 gene in a transgenic rat

Measurement of the amount of hHI7T213 gene expression in each organization was performed like the example 6. . That is, it is about the 11-weeks old transgenic rat (F1) of 96M. From a brain, the heart, the kidney, a spleen, liver, a retina, a lens, the skin, a small intestine, the stomach, muscles, and lungs to an example As 6 showed, total RNA was prepared, and TaqMan analysis was performed. At this time G3 PDH or an actin gene was used for the internal standard. In detection of G3 PDH, it is rodent. G3 PDH A set (Applied Biochemicals) is used and it is Fo in detection of an actin gene. rward primer

(5'-CGTGAAAAGATGACCCAGATCA-3' (array number: 18)), Reverse primer (5'-ACACAGCCTGGATGGCTACGTA-3' (array number: 19)) VIC indicator It is business about TaqMan primer (5'-TTTGAGACCTTCAACACCCCAGCCA-3' (array number: 20)). It was. Although hHI7T213 gene expression was accepted from any organ, the amount of manifestations is the heart. Many inclinations were suited with the kidney, a lens, the skin, and muscles ( drawing 5 ).

[Example 9]

[0133]

Pathology analysis and hHI7T213 gene expression of a transgenic rat

It sets to the main organs of a 5-weeks old rat, and is \*\* to pathology retrieval and hHI7T213 gene expression. Were and it investigated. Pathology analysis was performed as follows. The liver of a rat, the kidney, the heart, a spleen, lungs A

suprarenal gland, a testis, the ovary, and an eyeball are extracted, and it fixes with neutral-buffered-formalin liquid 10%. According to a conventional method, paraffin embedding is performed after \*\*, and it is 4. Thin sectioning was carried out by the thickness of mum. To a degree Microscopy was carried out after producing a hematoxylin-and-eosin (HE) stained specimen. In immobilization of an eyeball The processing for 30 minutes in Davidson fixing fluid is added before 10% neutral-buffered-formalin liquid processing. \*\*. hHI7T213 gene expression — In situ hybridization — it investigated by law. this — the time — DIG — an indicator — production of a RIBOPU lobe carried out was performed as follows. hHI7T21 The plasmid pCRScript 213-2 which inserted the fragment of 1.0 kbp containing three genes is produced. It carried out and imprinted after cutting with the restriction enzyme of 5' protrusion by T3 polymerase (antisense probe XhoI cutting was performed if carried out), or T7 polymerase ( and NotI cutting were performed as a sense probe). In hybridization, it is 25micro in thickness. The frozen section of m or the paraffin section with a thickness of 4 micrometers was used. It is 4% PA about an intercept. RAHORU marine-0.1 M Proteinase after fixing with a phosphate buffer solution (pH 7.4) K (1microg [ // ml ], Roche, 37-degree-C 10 minutes) processing, 0.2 M HCl processing The process for acetylation processing 10 minutes was performed for 10 minutes. A probe is added and it is at 55 degrees C. 50% formamide liquid containing 2xSSC performs 5microg [/ml ] RNaseA (NIPPON GENE) processing after washing by 55 \*\* after hybridization overnight, and it is the last concentration. 0.4xSSC washed at 55 degrees C. Detection of a signal was made to color in a black box in BCIP and NBA (Promega) overnight after anti-D IG-AP antibody (ROTSU SHU) processing. . The need is accepted and they are eosin (Wako) or Nuclear fast red (Funakoshi). It used and counterstain was performed.

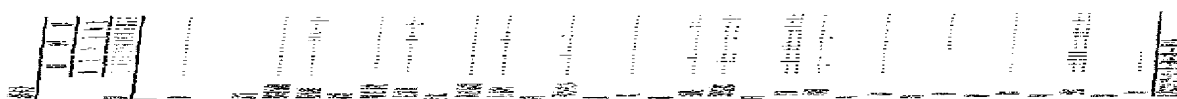
Inside [ in liver, the heart, a spleen, lungs, a suprarenal gland, a testis, and the ovary, abnormalities are accepted as a result of biopsy ] \*\*\*\* (Table 1).

[Table 1]

## 組織病理 - 個体観察 -

動物番号	96M, ♂										96M, ♀										13M, ♀							
	Non					Tg					Non					Tg					Non				Tg			
	1	2	3	7	8	9	12	13	14	16	17	18	19	20	21	22												
副腎	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
異常																												
眼																												
水晶体前極																												
融解/変性, 水晶体纖維						++																						
腫脹, 水晶体纖維						++																						
水晶体後極																												
融解/変性, 水晶体纖維						+																						
腫脹, 水晶体纖維						++																						
弓状領域						++																						
融解/変性, 水晶体纖維						+++																						
腫脹, 水晶体纖維						+++																						
網膜						+																						
網膜ロゼット/おりたたみ						P																						
心臓						-																						
異常						-																						
腎臓						-																						
拡張, 骨盤						P																						
尿管好塩基球増加						+																						
肝臓						+																						
髓外造血	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
単核球浸潤																												
肺						-																						
異常						-																						
卵巣																												
異常																												
皮膚																												
異常																												
脾臓																												
髓外造血	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
精巣																												
異常																												

検出の程度は次のとおりであった: -, なし; ±, 最小; +, 軽度; ++, 中程度; ++++, 顕著; P, あり。



in an eyeball, fusion/denaturation of the fibrae lentis accept -- having -- a typical cataract -- a diagnosis -- \*\*\*\* ( drawing 6 a-d). Moreover, with the kidney, the view of the increment in a basophilia renal tubule is acquired, and it is \*\* at the kidney. It was suggested that \*\*\*\*\* occurred or that the growth promotion activity of a tubular cell is high (drawing 7 a-b). furthermore -- the eyeball and the skin of a juvenile individual -- the above -- the same -- pathology retrieval -- line \*\*\*\* and time -- after-the-birth 3 to seven ages in day -- an eyeball -- also setting -- 5-weeks old o'clock -- the same -- being the abnormalities of the fibrae lentis accept -- having ( drawing 6 e-j) -- inside -- vortex lentis in the stella lentis iridica There is also an individual as which stratification is regarded ( drawing 6 g and h), and have shown the symptoms of a cataract from juvenile. I understand. Moreover, the view of the acanthosis and parakeratosis was acquired in the skin of a juvenile period ( drawing 8 a, c, e, g). hHI7T213 gene expression in the organ as which these abnormalities were regarded the place investigated by in situ hybridization -- an eyeball -- setting -- viviparous 12 age in day -- It was and the manifestation in the part which will become the fibrae lentis in the future was seen ( drawing 6 k and l). In the kidney A manifestation is seen by a renal tubule or the pulp rather than a glomerulus ( drawing 7 c-e), and it is a skin smell. As for \*\*, the manifestation was seen epidermis and near pore ( drawing 8 b, d, f, and h). These manifestation parts All were in agreement with the histological abnormality part in general.

[Example 10]

[0134]

The cataract and the amount of hHI7T213 gene expression in a transgenic rat It is hHI7T in order to clarify relation of hHI7T213 gene expression and the abnormalities in phenotype. It investigated about correlation of the amount of 213 gene expression, and the abnormalities in phenotype. Namely, 96M and 13 Individual used for the example 9 in TawMan analysis like the example 8 about M hHI7T213 gene expression which can be set was investigated, and it compared with extent of a cataract. The result It is shown in drawing 9 .

It compares with 13M and the directions of 96M with high extent of fusion/denaturation of the fibrae lentis are muscles. And there were many amounts of hHI7T213 gene expression in the skin. In moreover, the inside of the same network For the direction of an individual with high extent of a cataract, hHI7T213 gene expression is high \*\* relatively. \*\* was suited. As transgenic as the above results to a hHI7T 213 gene quantity manifestation The abnormalities in phenotype seen by the rat were able to consider possibility of correlating.

[Example 11]

[0135]

The abnormalities in epidermal differentiation seen by the transgenic rat Anti-keratin6 antibody (Funakoshi), anti-keratin14 antibody (Cosmobio), \*\* keratin10



antibody (DAKO), an anti-loricrin antibody (Funakoshi), anti-PCNA antibody Used immunity organization dyeing was performed as follows. That is, it is a xylene to a paraffin section. antigen unmasking solu after a sequence and an alcoholic sequence perform deparaffinization-ization It is ebullition after ebullition for 7 more minutes with a microwave oven in tion(Funakoshi)750 ml. It continued. To 100% acetone processing 5 minutes after returning to a room temperature over 1 hours or more Processing to depend was performed. the law of a vector stain kit (Funakoshi) — a method — blocking After various primary antibodies, a second antibody, 3% H2O2-PBS (5 minutes), and ABC reagent processing The DAB stain solution performed the coloring reaction (less than 10 minutes, Funakoshi). The need is accepted. Counterstain was carried out by Nuclear fast red (Funakoshi) or the hematoxylin. Anti-ke In ratin14 antibody, anti-keratin10 antibody, and anti-loricrin antibody dyeing, it is a section serial. It used. The result is shown in drawing 10 . keratin which is epidermis protein of relation in the contrast rat of seven ages in day at the time of a wound and growth The PCNA positivity cell which most 6 gene-expression cells are not seen, but is a growth related antigen is a table. It accepted only a hide basal layer and near pore ( drawing 10 d-f). It is 7 to it. By the epidermis part with much hHI7T213 gene expression of an age-in-day transgenic rat The increment in a keratin 6 gene-expression cell and the increment in a PCNA positivity cell are seen. \*\* ( drawing 10 j-l). Therefore, with the epidermis which hHI7T213 gene high-discovered, it is \*\*. It was suggested that \*\*\*\*\* promotion activity is accelerating.

Next, keratin 14 discovered by the basal cell layer in order to investigate the differentiation condition of epidermis, \*\* keratin 10 discovered by the \*\* \*\* stratum compactum, and loricrin \*\* discovered in a granulocyte layer The manifestation of \*\*\*\* was investigated. NonTg — keratin 14 and keratin 10 — and — abnormalities were not seen by the loricrin positivity cell — receiving ( drawing 10 a-c) — hHI an epidermis part with much 7T213 gene expression — keratin14 and keratin10 — \*\* and a loricrin positivity cell were increasing ( drawing 10 g-i).

From the above results, the acanthosis is keratin14 and keratin10, It reaches. Being connected with the increment in a loricrin positivity cell was suggested. It reaches keratin6. It follows on the increment in the clonogen of keratin14 positivity, and is the turnover time amount of a stratum-basale-epidermidis cell. It is in the condition in which the time amount which shortens, consequently enucleation-ization takes became inadequate, and the nucleus remained. Parakeratosis is seen and it is thought that it was observed as desquamation.

[Example 12]

[0136]

The epidermis free nerve ending seen by the transgenic rat

Duplex immunity organization using rabbit anti-PGP9.5 antibody and mouse anti-keratin6 antibody Chemistry investigated the epidermis free nerve ending. In a second antibody, it is the Alexa488 indicator anti-rabbit 2. Degree antibody and the Cy3 indicator anti-mouse second antibody were used. 25 micrometers in namely, thickness — fresh a frozen section — receiving — antigen unmasking solution(Funakoshi) 750ml — inside Ebullition was continued for 1 more minute after ebullition by \*\*\*\*\*. It applies for 1 hour or more, and is a room temperature.

After returning, processing [ / in acetone 5 minutes ] was performed 100%. It is BUROTSU to an intercept. After performing a king, a primary antibody, and second antibody processing, it observed in the laser beam microscope (LSM510, Zeis s). The result is shown in drawing 11 .

At the contrast rat ( drawing 11 a-c) of seven ages in day, keratin 6 positivity is most. It was not observed but many free nerve endings on epidermis were also accepted. It is keratin 6 to it. At 7 age-in-day transgenic rat with many positivities ( drawing 11 d-f), it is epidermis freedom. The synaptic ending was quite abundant as compared with the contrast rat. Moreover, not only epidermis but epidermis The free nerve ending was abundant also in the bottom as compared with the contrast rat ( drawing 11 g-i).

[Example 13]

[0137]

hHI7T 213 gene-expression analysis in a spinal ganglion

Having discovered hHI7T213 gene to a part of nerve cell which has the free nerve ending of a spinal ganglion is reported, and it is also considered that the abundant free nerve endings seen in the example 12 are as a result of the operation enhancement based on an overforeign gene manifestation in the spinal-nerves cell which hHI7T213 gene has originally discovered. In order to clarify this point, the introductory gene expression in the spinal ganglion of a transgenic rat was investigated first. A spinal ganglion, the skin, an eyeball, and the kidney were extracted from NonTg and the transgenic rat of resistance 18 age in day, it homogenized in ISOGEN (NIPPON GENE make), and total RNA was extracted with the conventional method. In the case of a spinal ganglion, it is total RNA 0.1. mug is used and, in the case of the skin, an eyeball, and the kidney, it is total RNA 1. mug was used, cDNA composition was performed according to the protocol using First strand cDNA synthesis kit (the Amersham Pharmacia Biotech K.K. make), and it considered as the template. The primer of TaqMan analysis (TaqMan7700, Applied Biochemicals) was shown in the example 6. For detection of hHI7T213 gene, namely, Forward primer (5'-TCCTGTCCGCTCTTAACAGCA-3' (array number: 17)), Reverse primer (5'-TTTTGACGCTGCCTAAAGGAG-3' (array number: 18)), And the primer set of the FAM indicator TaqMan primer (5'-TGCCAACCCCATCATTTACTTCTTCGTG-3' (array number: 19)) is used. In detection of G3 PDH of an internal standard, it is rodent G3 PDH. The set (Applied Biochemicals) was used. it is shown in drawing 12 -- as -- a spinal ganglion, the skin, an eyeball, and the kidney — hHI7T213 gene had discovered all.

[Example 14]

[0138]

The CGRP positivity synaptic ending of a transgenic rat

From an example 13, since hHI7T213 gene had also discovered the spinal ganglion of a transgenic rat, that a free nerve ending is abundant can consider the possibility of the operation enhancement by introductory gene quantity manifestation. if it originates in the operation enhancement based on the high manifestation by the spinal ganglion — epidermis — it is expected that only a free nerve ending becomes abundance specifically, and there is no change in the nerve fiber of the other types like the CGRP positivity nerve fiber also in any. In order to clarify this point and to

investigate the nerve-fiber distribution of those other than a free nerve ending, CGRP dyeing was doubled and was performed. At this time, the transgenic rat skin compared on appearance by both the normal specimen and the specimen which shows abnormalities, and clarifying also doubled whether it would be limited to the epidermis which is abnormal in whether change originates in a spinal ganglion, and it performed it.

The primary antibody used the Alexa488 indicator anti-rabbit second antibody and the Cy3 indicator anti-mouse second antibody for the second antibody using the rabbit anti-CGRP antibody, rabbit anti-PGP9.5 antibody, and mouse anti-keratin6 antibody. a fresh frozen section with a thickness of 25 micrometers — receiving — antigen unmasking solution(Funakoshi) 750ml — ebullition was continued for 1 more minute after ebullition with the microwave oven in inside. After returning to a room temperature over 1 hours or more, processing [ / in acetone 5 minutes ] was performed 100%. After performing blocking, a primary antibody, and second antibody processing to an intercept, it observed in the laser beam microscope (LSM510, Zeiss).

On the appearance of seven ages in day, on the normal transgenic rat skin, each of keratin 6 hot nodule is the same as that of NonTg, and abnormalities were not accepted. ( Drawing 12 a-f ). On the unusual transgenic rat skin, keratin 6 hot nodule and a PGP9.5 positivity fiber were quite abundant to it as compared with NonTg, and the CGRP positivity fiber was also abundant to coincidence ( drawing 12 g-i ). It also became clear that it was not specific to the free nerve ending which is carrying out the \*\* office of the phenomenon in which a synaptic ending becomes abundance by the transgenic rat to the abnormality part in epidermis from the above-mentioned results, and contains a PGP9.5 positivity fiber. Therefore, possibility of originating in the neurotrophic factor which is a certain inducer having come out from an abnormal epidermis side rather than originating in hHI7T213 gene carrying out the superfluous manifestation of the abnormalities in the skin in a transgenic rat by the spinal ganglion was suggested.

[Example 15]

[0139]

Manifestation enhancement of the neurotrophic factor group in the abnormality part in epidermis

On the transgenic rat skin which is abnormal in the example 14, since possibility that the neurotrophic factor was produced was suggested, NGF in epidermis and the amount of BDNF gene expression were investigated. The skin of a normal transgenic rat and an abnormal transgenic rat was extracted on NonTg and appearance, it homogenized in ISOGEN (NIPPON GENE make), and total RNA was extracted with the conventional method. total RNA 5 mug was used, cDNA composition was performed according to the protocol using First strand cDNA synthesis kit (the Amersham Pharmacia Biotech K.K. make), and it considered as the template. The following was used for the primer of TaqMan analysis (TaqMan7700, Applied Biochemicals). In detection of hHI7T213 gene, Forward primer (5'-TCCTGTCCGCTCTTAACAGCA-3' (array number: 17)), Reverse primer (5'-TTTTGACGCTGCCTAAAGGAG-3' (array number: 18)), The FAM indicator TaqMan primer (5'-TGCCAACCCCATCATTTACTTCTTCGTG-3' (array number: 19))

and for detection of a NGF gene Forward primer (5'-AGCCCACTGGACTAAACTTCAGC-3' (array number: 23)), Reverse primer (5'-GGGCACTGCGGGCTC-3' (array number: 24)), The FAM indicator TaqMan primer (5'-TTCCCTTGACACAGCCCTCCGC-3' (array number: 25)) and for detection of a BDNF gene Forward primer (5'-GGTGATGCTCAGCAGTCAAGT-3' (array number: 26)), Reverse primer (5'-CGAACCCTCATAGACATGTTTG-3' (array number: 27)), And a FAM indicator TaqMan primer (5'-TTTGAGACCTCCTCTGCTCTTTCTGC-3' (array number: 28) set is used. In detection of the actin gene of an internal standard, Forward primer (5'-CGTGAAAAGATGACCCAGATCA-3' (array number: 29)), Reverse primer (5'-GCACAGCCTGGATGGCTA-3' (array number: 30)), And the VIC indicator TaqMan primer (5'-TTTGAGACCTTCAACACCCAGCCA-3' (array number: 31)) was used. As shown in drawing 14 a, in the transgenic rat, the amount of hHI7T213 gene expression in the abnormal skin was more nearly intentionally [ than the amount of manifestations in the normal skin ] high. Moreover, as for NGF and the amount of BDNF gene expression, on the skin of an unusual transgenic rat, there were many amounts of manifestations between the amounts of manifestations in the skin of NonTg and a normal transgenic rat intentionally to there having been no difference ( drawing 14 b and c). From the above results, on the transgenic rat skin accompanied by the abnormalities in epidermal differentiation, since the amount of manifestations of neurotrophic factor groups, such as NGF and BDNF, increased, it was thought that the PGP9.5 positivity nerve fiber became abundance.

[Brief Description of the Drawings]

[0140]

[Drawing 1] The internality hHI7T213 gene expression in each organization of a mouse is shown. An upper panel expresses #8 gene expression and a lower panel expresses #11 gene expression, respectively. P0, E18, Ad, D, B, I, M, H, C, K, L, S, and T express a new born mouse, 18 age-in-day embryo mouse, adult mice, a dorsal spinal nerve root ganglion, a brain, a small intestine, muscles, the heart, a cortex, the kidney, liver, a spleen, and a testis, respectively.

[Drawing 2] It is the construction Fig. of expression vector pCAG 213-1 for transgenic rat creation.

[Drawing 3] The hHI7T213 gene expression in transgenic rat each network is shown. Non Tg is the experiment which used the non-transgenic rat, and Br, helium, and Ki express a brain, the heart, and the kidney, respectively. The error bar of a graph expresses a standard error.

[Drawing 4] The description of transgenic rat 96M is shown. (a) Left-hand side is a transgenic rat and right-hand side is a non-transgenic rat. An arrow head shows desquamation. (b) The result of the transgenic rat of five ages in day is shown. (c) The result of the non-transgenic rat of nine ages in day is shown. (d) The result of the transgenic rat of nine ages in day is shown.

[Drawing 5] Organization distribution of hHI7T213 gene in transgenic rat 96M is shown. Br, helium, Ki, Sp, Li, Re, Le, Sk, In, Mu, and Lu express a brain, the heart, the kidney, a spleen, liver, a retina, a lens, a small intestine, muscles, and lungs, respectively.

[Drawing 6] The result of the pathology analysis of the lens of transgenic rat 96M is shown. The result of the non-transgenic rat as contrast is expressed to a left-hand

side panel (a, c, e, f, i, k), and the result of 96M is expressed to a right-hand side panel (b, d, g, h, j, l). In 5W (a-d), 5 weeks old and P3 (e-j) show three ages in day, and E12 (k, l) shows viviparous 12 age in day.

[Drawing 7] The result of the pathology analysis in the kidney and hHI7T213 gene expression is shown. a and b are in situ of hHI7T213 gene [ in / for the result of the hematoxylin / eosine dyeing of the non-transgenic rat of five week \*\*, and a transgenic rat (H & E staining) / in c to e / the kidney of the non-transgenic rat (c) of three day \*\*, and a transgenic rat (d and e) ], respectively. The result of hybridization is expressed.

[Drawing 8] The acanthosis in a transgenic rat and the observation result of parakeratosis are shown. (a, b, e, f) And (c, d, g, h) the result of having used the non-transgenic rat and the transgenic rat is expressed, respectively. For (a, c, e, g), as a result of being a hematoxylin / eosine dyeing, (b, d, f, h) are in situ of hHI7T213 gene. The result of hybridization is expressed. (a-d) shows three ages in day, and (e-h) shows seven ages in day.

[Drawing 9] The amount of hHI7T213 gene expression in the muscles and the skin of transgenic rats 96M and 13M is shown. An individual number corresponds to Table 1.

[Drawing 10] The observation result of the abnormalities in epidermal differentiation in seven ages in day of transgenic rat 96M is shown. (From a to f) And (from g to l) the result of having used the non-transgenic rat and the transgenic rat is expressed, respectively. in situ high BURIDAOZESHON (f, l) of various antibody dyeing and hHI7T213 gene was written above.

[Drawing 11] The observation result of the epidermis free nerve ending in seven ages in day of transgenic rat 96M is shown. It is shown that Keratin6 and PGP9.5 use mouse anti-keratin 6 antibody and rabbit anti-PGP9.5 antibody, respectively. Moreover, Non (a-c) expressed the contrast rat (non-transgenic rat), Tg (d-i) expressed the transgenic rat, and the dotted line showed the boundary of epidermis.

[Drawing 12] The hHI7T213 gene expression in viviparous 18 age in day of transgenic rat 96M is shown. Non Tg is the experiment which used the non-transgenic rat, and D, S, E, and K express a spinal ganglion, the skin, an eyeball, and the kidney, respectively.

[Drawing 13] The observation result of the synaptic ending in after-the-birth 7 age in day of transgenic rat 96M is shown. It is shown that Keratin6, PGP9.5, and CGRP use mouse anti-keratin 6 antibody, rabbit anti-PGP9.5 antibody, and the rabbit anti-CGRP antibody, respectively. Moreover, as for Tg-Nor, on appearance, NonTg (a-c) shows a contrast rat (non-transgenic rat) for the skin of a normal transgenic rat, and Tg-Ab shows the unusual skin.

[Drawing 14] hHI7T213 gene in after-the-birth 7 age in day, the NGF gene, and BDNF gene expression of transgenic rat 96M are shown. As for Tg-Nor, on appearance, NonTg (a-c) shows a contrast rat (non-transgenic rat) for the skin of a normal transgenic rat, and Tg-Ab shows the unusual skin. A value is an average value of  $n=4$  and SE shows an error bar (for t-TEST: \*\*,  $p<0.01$  and \*\*\* are  $p<0.001$ ).

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[Translation done.]

**\* NOTICES \***

**JPO and NCIPi are not responsible for any damages caused by the use of this translation.**

- 1.This document has been translated by computer. So the translation may not reflect the original precisely.
- 2.\*\*\*\* shows the word which can not be translated.
- 3.In the drawings, any words are not translated.

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**DESCRIPTION OF DRAWINGS**

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